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REPRODUCTIVE BIOLOGY AND BIOLOGICAL RHYTHMS IN *Arvicanthis niloticus*

By

Teresa L. McElhinny

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ABSTRACT

REPRODUCTIVE BIOLOGY AND BIOLOGICAL RHYTHMS IN
Arvicanthis niloticus

By

Teresa L. McElhinny

Arvicanthis niloticus are small-bodied, murid rodents that inhabit sub-Saharan Africa. The taxonomy of the genus *Arvicanthis* is the source of much debate: some authors recognize just one species, whereas others describe up to six species. The biology of *A. niloticus* is poorly understood, as the species has been little studied, therefore the purpose of the work presented in this thesis was to elucidate aspects of the reproductive biology and biological rhythms of captive *A. niloticus*. My data suggested that multiparous female *A. niloticus* ovulate spontaneously, at intervals of 5-7 days. The mean duration of the gestation period was 24.25 days. This species exhibits a copulatory pattern similar to other murids. I found that *A. niloticus* are diurnal with respect to general activity and core body temperature rhythms, although peaks in activity and temperature occur at dawn and dusk. Copulation tends to occur during the morning peak of activity, while parturition occurs outside the times of peak activity.

To Pat

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PREFACE

The most common mammals on earth are short lived, herbivorous rodents living in tropical habitats. The Order Rodentia contains 40% of all living mammals, and four to five times as many species of mammals live in the tropics as are found in the rest of the world (Nowak, 1991). Despite the diversity and ecological importance of tropical rodents, little is known about their reproductive biology. Most research in this area has involved the use of domesticated laboratory species from temperate regions, and has not reflected the importance or diversity of the group. In the interest of furthering the study of reproductive biology in mammals, and to ensure that we account for the diversity that exists, more consideration should be given to tropical mammals, and in particular, to tropical rodents.

I have studied several behavioral and physiological aspects of reproductive biology, as well as circadian rhythms of temperature and activity, in the female of one tropical rodent species, *Arvicanthis niloticus* (Figure 1). *A. niloticus* are herbivorous rodents which inhabit tropical Africa. With their blunt faces and stocky bodies, *A. niloticus* are physically similar to voles (Kingdon, 1974; Carleton and Musser, 1984), although they are larger, and have longer tails and more prominent pinnae. This species is an excellent candidate for laboratory study because it is handled fairly easily, and it breeds well in captivity. Relatively low levels of aggression allow animals of the same

Figure 1. *Arvicanthis niloticus* (Rosevear 1969).

sex to be housed together, and males can remain housed with their mates without danger of pups being cannibalized. Behavioral study of *A. niloticus* is facilitated by their tendency to be active during hours of daylight.

This thesis is divided into chapters which address the specific topics of my research. Chapter I is a review of the literature concerning *Arvicanthis*, including taxonomy, systematics, ecology, and reproductive biology. In Chapter II, I discuss experiments that I performed in order to elucidate the estrous cycle in *A. niloticus*. These include vaginal smears, paired encounters between males and females, study of male responses to female odors, and analysis of circadian rhythms in the female. Chapter II also contains discussion of an experiment in which I examined the ovaries of females of different ages and levels of sexual experience in order to determine whether *A. niloticus* ovulate spontaneously or reflexively. In Chapter III, I present work in which I used video analysis to describe the morphology of copulation in this species, to elucidate timing of parturition, and to calculate a precise length of the gestation period. In Chapter IV, I present work which describes several biological rhythms of *A. niloticus*. I explored the

circadian rhythmicity of this species, describing rhythms of temperature, gross motor activity, and wheel-running. I also looked at the timing of copulation and parturition in relation to circadian activity and temperature rhythms.

CHAPTER I

ARIVCANTHIS NILOTICUS NATURAL HISTORY

The purpose of this chapter is to present a review of existing information about the genus *Arvicanthis* in general, and about *Arvicanthis niloticus* in particular. In subsequent chapters I will attempt to add to this knowledge pool by describing my own work involving the reproductive biology and biological rhythms of *A. niloticus*.

I.1 DISTRIBUTION AND HABITAT

Arvicanthis (Lesson, 1842) is a tropical genus, inhabiting the Nile valley, the southwestern part of the Arabian peninsula (Harrison, 1972), and much of Africa from Senegal to Somalia (Kingdon, 1974), south of the Sahara and north of the Zambezi river (Neal, 1981). The common name, the Nile grass rat, refers to the fact that the first specimen known to Europeans came from the Nile Valley (Rosevear, 1969). While the range of *Arvicanthis* spans the continent, the members of this genus occur most commonly in East Africa (Kingdon, 1974). Specifically, species belonging to this genus occur to the west in Mauritania (Musser and Carleton, 1993), Senegal (Rousseau, 1983), Mali (Rousseau, 1983), Burkina Faso (Rousseau, 1983; Kaminski *et al*, 1984), Gambia (Kaminski *et al*, 1987), Guinea, Sierra Leone (Harrison, 1972), Ivory Coast (Musser and Carleton, 1993), Ghana (Harrison, 1972), Togo, and Benin (Musser and Carleton, 1993).

In central Africa *Arvicanthis* have been found in Niger (Rousseau, 1983), Chad (Rousseau, 1983), Nigeria (Harrison, 1972), and the Central African Republic (Matthey, 1965; Rousseau, 1983). In northeastern Africa they occur in Egypt (Kaminski *et al*, 1984), Sudan (Rousseau, 1983), Ethiopia (Corbet and Yalden, 1972; Dorst, 1972; Yalden et al, 1976; Yalden, 1988), and the Somali Republic (Drake-Brockman, 1910). On the Arabian peninsula they have been found in Yemen (Harrison, 1972). In eastern Africa they occur in Kenya (Coe, 1973; Kingdon, 1974), Uganda (Bere, 1962; Delany and Neal, 1966; Delany, 1975), Tanzania (Allen and Loveridge, 1933; Kingdon, 1974; Packer, 1983; Senzota, 1983), Burundi, Zaire (Musser and Carleton, 1993), and south into Zambia (Ansell, 1960; Ansell, 1978) (Figure 2).

The distribution of *Arvicanthis* within the countries mentioned above (Figure 3) is dictated by the type of vegetation present. The preferred habitat of *Arvicanthis* is described as dry savannas, woodlands and grasslands (Kingdon, 1974). *Arvicanthis* are found in the northern and central Sudan along rivers and canals (Schmutterer, 1969), and Delany (1986) observed *A. niloticus* living along river edges in Kenya. *Arvicanthis* are also associated with water to the north where their range runs along the Nile in Egypt. In areas where the species is commensal with man, they can be found in cultivated land, native huts and grain stores (Delany and Neal, 1966). *A. niloticus* appear to be limited in distribution by dense forest and desert (Poulet and Poupon, 1978). Comparing the species distribution map in Figure 3 with the vegetation map in Figure 4, one can see that *Arvicanthis* prefer open woodlands and savannas, but avoid the Sahara and Sudanese deserts to the north, as well as the wet lowland forest of the Congo Basin in central Africa.

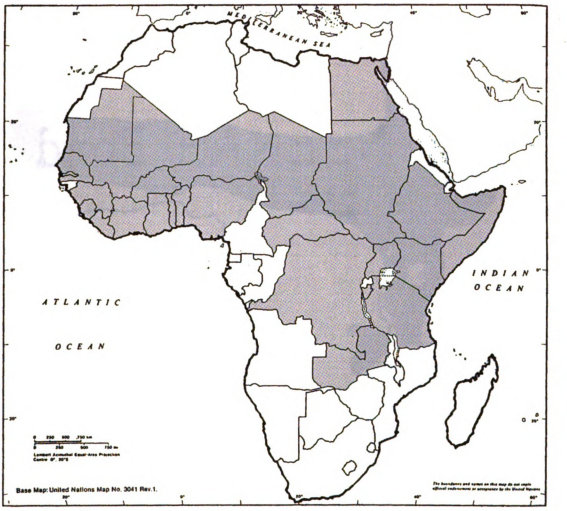


Figure 2. African countries in which *Arvicanthis* are known to occur (base map from *Africa Today, an Atlas of Reproducible Pages.* 1983).

Figure 3. Species range of *Arvicanthis niloticus* (Kingdon, 1974).

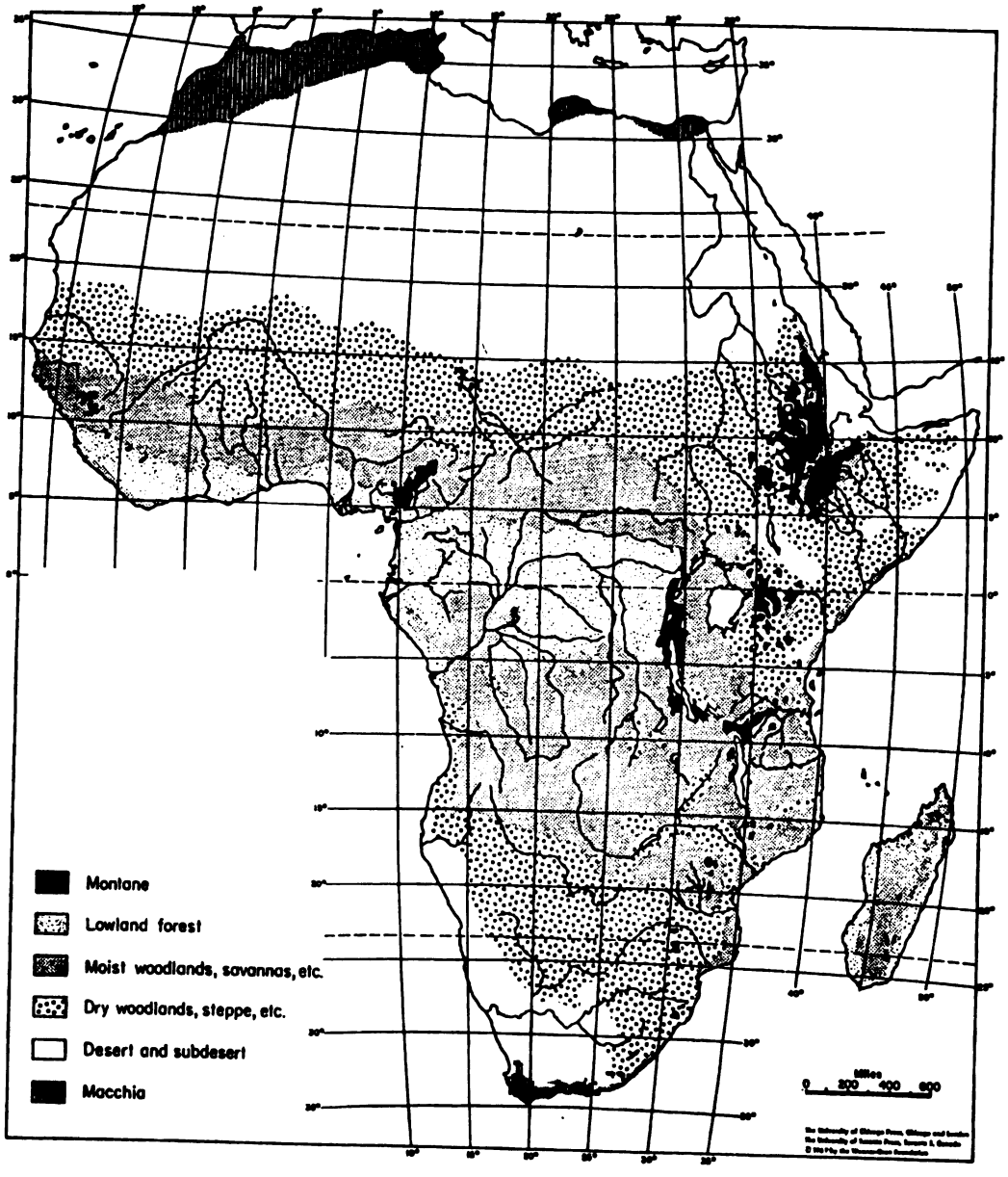


Figure 4. Simplified vegetation of Africa (*Africa Today, an Atlas of Reproducible Pages.* 1983).

I.2 TAXONOMY AND SYSTEMATICS

Rats of the genus *Arvicanthis* are ground dwelling rodents of the family Muridae, subfamily Murinae. Murinae is the largest subfamily within the Muridae, and represents a very heterogeneous group. Graur (1994) suggested that neither Muridae nor Murinae is monophyletic, and that these taxonomic groups are in need of revision. Chromosomal phylogenetics (Viegas-Pequignot *et al*, 1983; Chevret *et al*, 1993) and sperm morphology (Baskevich and Lavrenchenko, 1995) have shown that, although *Arvicanthis* and *Rattus* occur in the same subfamily, they are probably rather distant relatives. Viegas-Pequignot *et al.* (1983) suggested a "dichotomic evolution" (p. 276) for the two groups from which the genera *Arvicanthis* and *Rattus* arise. The electrophoretic studies of Bonhomme *et al.* (1985) showed that the 16 African murid genera (including *Arvicanthis*) they analyzed form a monophyletic subgroup separate from *Rattus* and *Mus*. Within Africa, *Arvicanthis* are generally allied with the other herbivorous murids, based on dental and cranial characteristics (Misonne, 1969). These other herbivorous murids are: *Pelomys*, *Mylomys*, *Hybomys*, *Lemniscomys*, *Rhabdomys*, and *Dasymys* (Kingdon, 1974). *Arvicanthis* are distinguished from other African murid genera by their relatively dark, coarse pelage and smooth incisors.

Taxonomic relationships within the genus Arvicanthis are poorly understood, with 17 to 37 forms recorded by early workers (Dollman, 1911; Allen, 1939; Ellerman, 1940). Authors today suggest the existence of from one (Rosevear, 1969; Misonne, 1971; Honacki *et al*, 1982) to five (Nowak, 1991; Musser and Carleton, 1993) distinct species. The most recent treatment of the genus is by Musser and Carleton (1993). These authors recognize 5 species: one (*A. niloticus*) ranges across Africa from Senegal to Ethiopia,

north to Egypt and South to Zambia, two species (*A. abyssinicus* and *A. blicki*) are endemic to Ethiopia, and two Eastern African species (*A. nairobae* and *A. somalicus*) occur within, and east of, the Rift Valley. There is some disagreement in the literature regarding the scientific name of the most widespread species of *Arvicanthis*: some investigators refer to it as *A. niloticus* (e.g. Musser and Carleton, 1993), while others prefer *A. abyssinicus* (e.g. Nowak, 1991).

There has been a recent effort to revise the taxonomic status of *Arvicanthis* through analysis of blood proteins and chromosome number. Kaminski *et al.* (1984) and Kaminski and Petter (1984) studied the blood proteins albumin, transferrin, esterase and 6-phosphogluconate dehydrogenase in wild-caught animals from three African countries: Senegal, Burkina Faso and Egypt. Kaminski *et al.* (1984) found inter-population differences and intra-population genetic polymorphism. Kaminski *et al.* (1984) concluded that at least 3 forms could be recognized based on structural differences among the proteins examined. The authors did not interpret this as evidence for speciation, but rather as a step toward speciation. The polymorphisms in blood protein composition, found between populations in east and west Africa, highlight the plasticity of *Arvicanthis*, and may indicate that further speciation is in progress within this genus (Kaminski *et al.* 1984).

Kaminski *et al.* (1984) successfully bred a female *Arvicanthis* from Senegal with a male from Cairo to produce four successful litters. Petter *et al.* (1969) were also successful in breeding a series of mating couples crossed from Senegal and Ethiopia. Production of offspring by pairing animals from such distant localities strengthens the argument of Musser and Carleton (1993) that one species inhabits the huge geographic

area shaded in Figure 3.

Kaminski *et al.* (1987) further analyzed the blood proteins of 152 *Arvicanthis* from 11 localities in Senegal. They found polymorphisms among animals from the northern and southern localities. Their northern collecting sites were along the Senegal river and along Cape Vert. There were only two southern collecting sites, at Cape Skirring in Gambia, and Kedougou in southeastern Senegal. These authors hypothesized that the animals from the south were similar to the specimens they had previously studied from Burkina Faso (Kaminski *et al*, 1984). Kaminski et al. (1987) determined that together these animals from southern Senegal, Gambia, and Burkina Faso differed from those from northern Senegal, constituting a species different from *Arvicanthis niloticus*. Thus, two species may exist in western Africa, *A. niloticus* to the north in Senegal, and another species to the south and east in Gambia and Burkina Faso. Kaminski *et al.* (1987) found no major reproductive barrier between the two groups in the natural environment, but stated that Petter unsuccessfully attempted to breed animals from Senegal and Burkina Faso. The authors did not state whether they believed these two forms to be sympatric in western Africa.

The first studies of chromosome number in *Arvicanthis* were performed by Matthey in 1959 and 1965 (see Table 1). Matthey (1959) described the first specimen as *A. abyssinicus*, and reported a karyotype of 62, but did not report a collection site. Matthey's (1965) second specimen, described as *A. niloticus*, was collected in the Central African Republic, and its karyotype was reported as 56 (Figure 5). Veigas-Pequignot *et al.* (1983) reported the karyotype of an *Arvicanthis* from Cairo as 62 (n= 1 individual). In

Table 1. Karyotypes of *Arvicanthis* from different geographic regions in Africa.

Investigator	Geographic Region	Karyotype
Matthey (1959)	?	62
Matthey (1965)	Bangui, Central African Republic	56
Viegas-Paquignot *et al.* (1983)	Cairo, Egypt	62
Capanna *et al.* (1985)	Afgoi, Somalia	44
Volobouev *et al.* (1987)	Lakoumbala, Central African Republic	58
Volobouev *et al.* (1988)	Bamako, Mali	62
Volobouev *et al.* (1988)	Oursi, Burkina Faso	62
Volobouev *et al.* (1988)	Brancon, Senegal	62
Orlov (1990)	Gambella, Ethiopia	62
	Ambo, Ethiopia	62
	Awash River Valley, Ethiopia	56
	Gamo-Gofo, Ethiopia	60

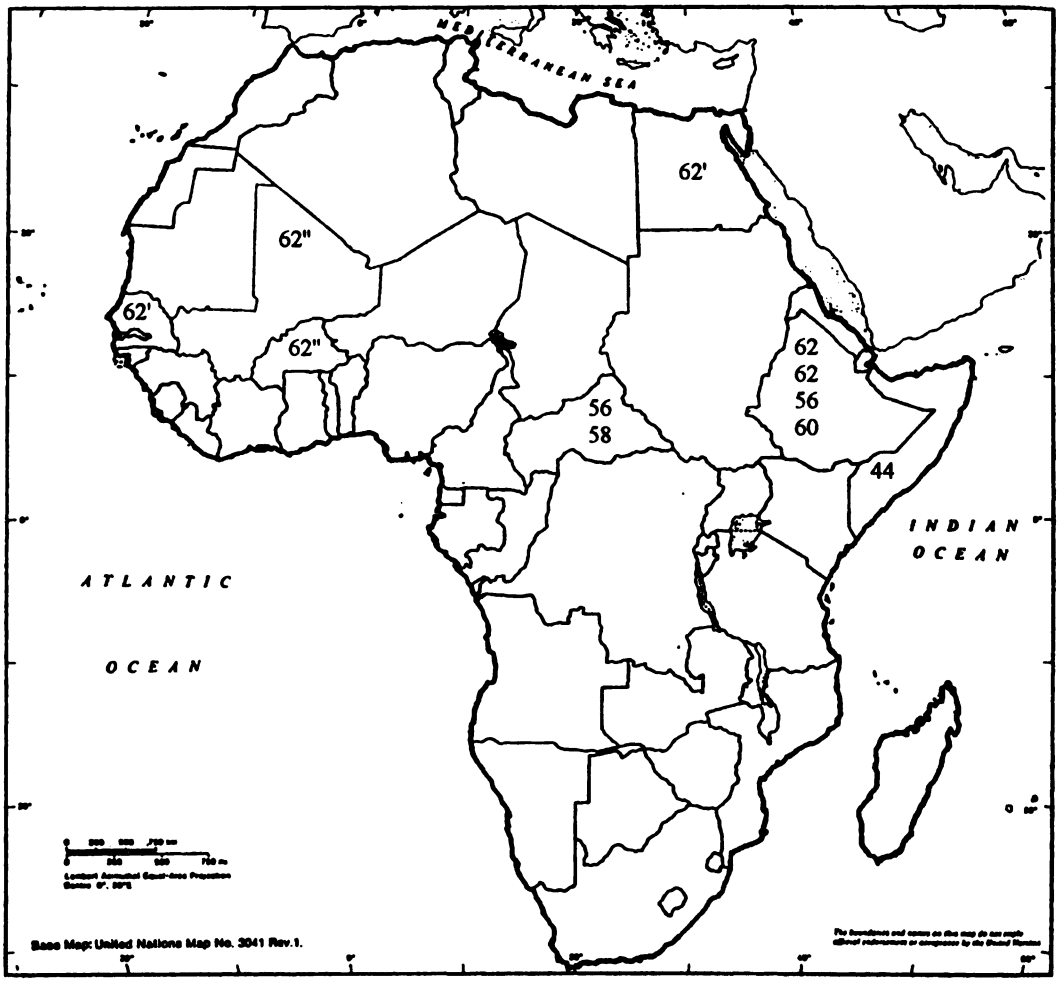


Figure 5. Distribution of described karyotypes of *Arvicanthis* across Africa. X′ and X″ karyotypes indicate the same species in different geographic areas (base map from *Africa Today, an Atlas of Reproducible Pages.* 1983).

1987, Volobouev *et al.* compared this number with that of an individual from the Central African Republic, which was 58 (n= 1). Based on differences in chromosome number and chromosomal structure, the authors concluded that at least two distinct species existed in Africa. Capanna and Civitelli (1988) found that a single specimen of *Arvicanthis* from Somalia, which they called *A. niloticus*, displayed a karyotype of 44. These authors concluded that each of the karyotypically differentiated populations mentioned above should be considered a separate species. Capanna and Civitelli (1988) hypothesized that the description of animals with 56 (Matthey, 1965) and 58 (Volobouev *et al*, 1987) chromosomes in the Central African Republic may be the result of the presence of an intermediate evolutionary stage of balanced chromosomal polymorphism. As there is no further information available, I will consider the animals from the Central African Republic to represent one species, different from *A. niloticus*.

Volobouev et al. (1988) examined animals from Egypt (n=1), Senegal (n= 1), Mali (n= 1), and Burkina Faso (n= 1), and proposed that at least 3 species of *Arvicanthis* exist across Africa. From their sample, these investigators found three groups of animals with identical karyotypes from 1) Egypt and Senegal (karyotype= 62), 2) Burkina Faso and Mali (karyotype= 62), and 3) the Central African Republic (karyotype=58). While the animals from the first two groups described have the same number of chromosomes, they are considered different karyotypes because the positions of the chromosomal centromeres are different. In animals from Egypt and Senegal, all but one pair of chromosomes were acrocentric, while in animals from Burkina Faso and Mali, only 22 pair were acrocentric, and the rest were metacentric and submetacentric. Volobouev *et al.* (1988) concluded that the karyotypes of these animals were adequately different to merit

classification as three distinct species. This would not conflict with results of the cross-breeding experiments mentioned above (Kaminski *et al*, 1984, 1987), since Volobouev *et al.* (1988) considered animals from Senegal and Egypt to represent the same species of *Arvicanthis*, different from the species found in Burkina Faso. Volobouev *et al.* (1988) proposed that animals from Ethiopia are probably of the same species as those from Senegal and Egypt, since Petter *et al.* (1969) were able to successfully cross animals from Senegal and Ethiopia. Volobouev *et al.* (1988) attempted to relate the three species that they found to forms previously described which are reported to have similar geographic distributions, but concluded that a more complete study is needed to establish the distribution limits of each species. The taxonomy proposed by Volobouev *et al.* (1988) is as follows: one species (*A. niloticus*) occurs in Senegal, Egypt and possibly Ethiopia, a second (*A. centralis*) in the Central African Republic, and a third species (*A. solatus*) in Burkina Faso and Mali.

The results reported by Volobouev *et al.* (1988) suggest that three species of *Arvicanthis* exist in West Africa (*A. niloticus*, *A. centralis* and *A. solatus*), which would increase the total number of species of *Arvicanthis* in Africa to seven, including species based on morphological characteristics: the two Ethiopian endemics (*A. abyssinicus* and *A. blicki*) and the two species present east of the Rift Valley (*A. nairobae* and *A. somalicus*). Capanna and Civitelli (1988) maintain that their specimen from Somalia (2n=44) is *A. niloticus*, not *A. somalicus*, which would further divide *A. niloticus* (2n=62, as per Volobouev *et al*, 1988), and may bring the grand total of species within the genus to eight.

The most recent treatment of chromosome number in *Arvicanthis* comes from

Orlov *et al.* (1990), in which the authors examined animals from four different regions in Ethiopia. They found that all four areas yielded animals with karyotypes unique to one another. Two groups possessed karyotypes of 62 chromosomes, yet the morphology of the chromosomes was different enough that the authors classified them as different species (*A. abyssinicus* and *A. dembeensis*). This classification is supported by analysis of cranial morphology (Bekele *et al,* 1993). The other two groups described by Orlov *et al.* (1990), with chromosome numbers of 56 and 60, were not classified. It is apparent from the chromosome numbers however, that these two animals are species distinct from one another, and from the aforementioned *A. abyssinicus* and *A. dembeensis*. Although the taxonomy of *Arvicanthis* in Ethiopia remains puzzling, it is clear that the inclusion of these animals in the taxonomy would put the total number of species in the genus *Arvicanthis* above eight.

Kingdon (1974) indicated that a subfossil of *Arvicanthis* was found in Palestine, and Capanna and Civitelli (1988) hypothesized that *Arvicanthis* colonized Africa through a southern and southwesterly spread from Egypt. *Arvicanthis* are known from Algeria in the Middle Pleistocene and the Holocene. Arvicanthis are thought to have migrated through the Sahara and possibly along the Atlantic coast during the Middle Pleistocene (Kowalski and Rzebik-Kowalska, 1991). Consequently, Capanna and Civitelli (1988) suggested that the karyotype of the Egyptian animals (2n = 62; Viegas-Paquignot *et al*, 1983) is ancestral, and that the differences in chromosome number seen today are a result of changes that occurred during the southerly range expansion across Africa.

The biological species concept describes a species as a group of interbreeding or potentially interbreeding populations of individuals incapable of breeding with

individuals from other such populations (Mayr, 1942). In the future, it would be prudent to perform cross-breeding experiments, in addition to exploring the distribution patterns of each of the *Arvicanthis* groups described here. This information would determine whether description of these groups as species is warranted, according to the biological species concept.

There is currently very little information available regarding blood proteins or karyotypes from *Arvicanthis* captured in eastern Africa south of Egypt and Ethiopia. Thus, the morphologically based taxonomy of Musser and Carleton (1993) of *Arvicanthis* in eastern Africa remains unmodified. The animals used in the experiments reported here were captured at two locations west of the Rift Valley, and preliminary results from sequencing of the Sry gene of these animals indicate that they are different from *A. nairobae* (Barbara Lundigran, personal communication). Therefore, for the purpose of this dissertation I will follow Musser and Carleton (1993) in assuming that the only species of *Arvicanthis* present in Kenya west of the Rift Valley is *Arvicanthis niloticus*, a species which apparently ranges across Africa, and exhibits a great amount of intra- and interpopulation morphological variation. Some of the information reported in this chapter may refer to other species of *Arvicanthis* according to recent reorganization of the genus reviewed here. Therefore, I have indicated the collecting location or study site for locations outside the east African countries of Kenya, Uganda and Tanzania,.

I.3 PHYSICAL CHARACTERISTICS

Arvicanthis have been compared to the microtine rodents with respect to body conformation (Carleton and Musser, 1984), and they do generally resemble large voles,

but with longer, thicker tails and more prominent pinnae. As noted earlier, *A. niloticus* have coarse, dark fur on their dorsal surfaces, and lighter fur on the belly. On both the fore and hind feet, the second, third and fourth phalanges are considerably longer than the first and fifth phalanges. The tail is fairly well haired. The ears are usually reddish, and are fairly large and rounded. Females have six mammae. The incisors are ungrooved, in contrast to those of the similar genus, *Mylomys* (Rosevear, 1969). The periosteum covering the cranium is darkly pigmented with melanin, apparently serving to absorb solar radiation (Carleton and Musser, 1984). Kingdon's (1974) mean length measurements for adult *Arvicanthis* in east Africa are as follows: head and body: 106-204 mm; tail: 100-152 mm; hind foot: 23-32 mm; and weight 50-120 g. In an analysis of morphological variation of *Arvicanthis* across Africa, Rousseau (1983) found that the most noticeable differences among the animals sampled were in body size. There was a west-to-east cline in body size, with the animals in the east being the smallest.

I.4 ECOLOGY

I.4.a Diet

Data from fecal analysis, stomach content analysis, and direct observation of foraging have revealed that the diet of *A. niloticus* consists primarily of leaves and stems of herbaceous vegetable matter (both monocotyledons and dicotyledons), supplemented with insects, seeds and fruits (Delany, 1964; Delany and Neal, 1966; Coe, 1972; Nandwa, 1973; Neal, 1981; Rabiu and Fisher, 1989; Delany and Monro, 1986; K. E. Holekamp, personal communication). Gautun et al. (1985) noted that during periods of high

population density, *Arvicanthis* in Senegal would resort to feeding on the bark of *Acacia* trees, causing considerable damage. Rabiu and Fisher (1989) reported that the diets of males and females were similar. Grasses predominated in the diet during the dry season (Rabiu and Fisher, 1989; Neal, 1981), and this prevalence continued into the early rainy season (Taylor and Green, 1976; Delany and Monro, 1986). Seeds were taken as they began to appear during the rainy season (Taylor and Green, 1976; Rabiu and Fisher, 1989), and continued into the beginning of the dry season (Delany and Monro, 1986; Neal, 1981).

Rabiu and Fisher (1989) noted that in general *Arvicanthis* in Nigeria tended to become more omnivorous with the commencement of the rains, presumably because it was during the rains that more variation in food types became available. Seeds were preferred over grass leaves when both foods were present in (Delany and Monro, 1986). Delany and Kansiimeruhanga (1970) tested wild-caught *Arvicanthis* from Uganda for food preferences using seeds, insects and a variety of vegetation types. The animals preferred cassava and wheat kernels over grasses, insects or the leaves of coffee, sweet potato and *Eucalyptus*. Senzota (1982) referred to *A. niloticus* as opportunistic feeders because the extent to which a given species of grass was clipped by the rats was linearly related to the abundance of the species. He also reported that the chance of a particular grass being eaten increased with its proximity to an *A. niloticus* burrow system. All grass species within the animals' foraging area were eaten. The foraging radius was up to seven meters around an *A. niloticus* colony in the Serengeti, with the radius depending on the abundance of grasses (Senzota, 1983). Packer (1983) reported that the average foraging distance from burrows in his Serengeti study site was six meters.

Senzota (1983) has suggested that there is resource partitioning between *A. niloticus* and savanna ungulates such as wildebeest (*Connochaetes taurinus*) and Thomson's gazelles (*Gazella thomsonii*). He observed that in Tanzania, where the diets of the two groups overlap somewhat, the rats eat species of grass that the ungulates tend to avoid. Senzota (1983) further noted that *A. niloticus* prefer the stems of the grasses, ripping them apart with their claws and teeth, discarding the leaves. Ruminant ungulates prefer to take the leaves, as the stems are harder for them to digest. Senzota (1983) concluded that by eating species and parts of the grasses that ungulates tend to avoid, *A. niloticus* may have facilitated resource packing in this part of the Serengeti ecosystem.

I.4.b *A. niloticus* as a Pest

A. niloticus populations have been known to reach remarkably high densities, and also to crash periodically (Delany and Monro, 1985b). Animal numbers in dry bush savanna during a population explosion in Senegal went from 0/ha to 100/ha in a matter of months (Poulet, 1972). During an *A. niloticus* outbreak in Tanzania associated with burning of the savanna, *A. niloticus* numbers were concentrated such that "one could hardly avoid stepping on them" (p. 426, Hubbard, 1972). The dietary preference for seeds shown by *A. niloticus* has resulted in their classification as a pest species in areas of Africa where cereal crops are grown. Dieterlen (1990) reported that *A. niloticus* is Number One on Egypt's "most wanted" list, and that the species may have been responsible for rat plagues dating back to the time of the Pharaohs. A massive explosion of *A. niloticus* populations in Kenya and Tanzania in 1951 and 1962 resulted in damage to corn, wheat and barley crops. Also in 1962, *A. niloticus* were responsible for damage

to cotton crops in Sudan, disturbing seedlings as well as mature bolls during the harvest period (Ripper and George, 1965; Taylor, 1968). Schmutterer (1969) described *A. niloticus* as minor pests in the Sudan, gaining major pest status in certain years. In the Sudan they have attacked dura (a cereal), pennisetum (a grass), maize, wheat, millet, groundnuts, sesame, haricot beans, tomatoes, stored cereal products of various kinds, and guava and mango trees when fruiting (Ripper and George, 1965; Schmutterer, 1969). *A. niloticus* were found eating coffee, cassava, and sweet potato on farms in Uganda (Delany and Kansiimeruhanga, 1970). During a 1975-1976 outbreak in Senegal, *A. niloticus* attacked rice, sorghum, millet, and cassava (Myllymäki, 1979), and stripped bark and shoots from *Acacia* trees, causing damage to 80% of the trees in one km^2 study area. *A. niloticus* displayed adaptive behavior in response to these unusually high population densities. At such times these animals not only invaded the arid habitats that they usually avoid, but they also climbed trees in order to forage (Poulet and Poupon, 1978).

Measures to control *A. niloticus* numbers in Sudan and Kenya have included baiting them with the poisons Warfarin, zinc phosphide (Ripper and George, 1965; Schmutterer, 1969), and Endrin (Taylor, 1968).

A. niloticus have been identified as a carrier of plague (Davis, 1962; Hubbard, 1972), and Nairobi sheep disease, a tick-borne virus that affects sheep and goats (Cox, 1979). They are highly susceptible to Rift Valley fever (Davis, 1962), a mosquito-borne virus which causes serious disease in lambs, and occasionally infects humans (Cox, 1979). Therefore, the population explosions mentioned above could potentially raise health concerns for sympatric human populations, in addition to concerns about their documented effects on agriculture.

Arvicanthis outbreaks are reminiscent of population explosions of microtine rodents in northern temperate zones, since they cause damage to cereal crops, and *Microtus aggrestis* has been known to strip the bark from trees (Myllymäki, 1979). However, abrupt increases in the numbers of *Arvicanthis* do not occur on an annual or cyclical basis (Taylor, 1968) as they do among the microtines (for a review, see Southern, 1979). Population increases of *Arvicanthis* are believed to be caused by unusually high rainfall, which increases food availability, and in turn lengthens the breeding season (Taylor, 1968; Poulet and Poupon, 1978; Sicard et al, 1994). This simple cause-effect relationship involving increased food availability has been rejected by those who study microtines (Krebs, 1996). Fluctuations in numbers of voles and lemmings are believed to be caused by an interaction of intrinsic effects of spacing behavior (affecting dispersal, mortality and reproduction) and extrinsic effects, most likely predation and food availability (Krebs, 1996). This explanation of microtine population cycles might also apply to *A. niloticus*, but more detailed observations of *Arvicanthis* population outbreaks are required to determine whether this is true. Most of the reports involving massive increases in *Arvicanthis* numbers have focused on the resulting damage to crops rather than on the dynamics of the rodent populations involved. One exception is Poulet's (1976) description of the 1975 population surge in Senegal. A severe drought in 1972, followed by steadily increasing annual rainfall, led to dramatically increased population numbers of several rodent species in 1975-1976. High population densities led to the migration of *A. niloticus* from their normal habitat in nomad campsites and cultivated areas to the less desirable dry savanna. This opportunistic dispersal may be an important feature of spacing behavior in this species. Once established in the dry savanna,

immigrant rats reproduced quite rapidly, and population densities soon exceeded habitat carrying capacities, resulting in starvation and high mortality. Mortality was increased by predation due to abnormally high concentrations of diurnal Palearctic birds of prey. Thus, this outbreak of animals was affected by the extrinsic factors of food availability and predation, and spacing behavior in the form of dispersal to less populated areas, increased reproduction after dispersal, and mortality due to famine.

I.4.c Predators

Indigenous predators in the natural habitat of *A. niloticus* include spitting cobras (*Naja nigricolis*), black-backed jackals (*Canis mesomelas*), long crested hawk eagles (*Lophoerus occipitalis*), black shouldered kites (*Elanus caeruleus*), black headed herons (*Ardea melanocephala*) (Senzota, 1990), dwarf mongooses (*Helogale parvula*) (Packer, 1983), and native Africans (*Homo sapiens*) (Vesey-Fitzgerald, 1966).

I.4.d Activity

In their natural habitat, *A. niloticus* have generally been observed to be diurnal (Quilici et al, 1969; Delany and Kansiimeruhanga, 1970; Kingdon, 1974; Packer, 1983; Rabiu and Fisher, 1989), or diurnal with crepuscular tendencies (Senzota, 1990; see also Katona and Smale, in press). Figure 6 shows an actogram for *A. niloticus* from Delany and Kansiimeruhanga (1970). These data are from direct observations of movement and feeding of a caged, wild-caught animal kept in its natural photoperiod. The figure clearly shows that the animal was more active during the day. However, some investigators consider *A. niloticus* to be partially nocturnal (Ansell, 1960; Delany and

Neal, 1966;

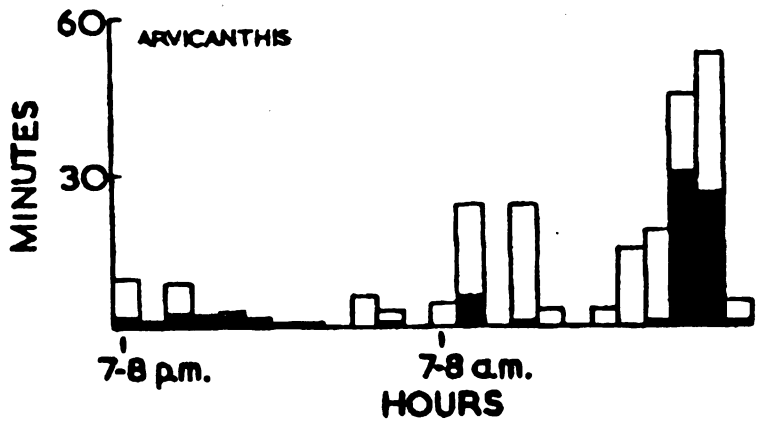


Figure 6. Activity rhythm of an individual *Arvicanthis niloticus* from Uganda. Time active is indicated by the open bars, and time spent feeding by the closed bars (Delany and Kansiimeruhanga, 1970).

Vesey-Fitzgerald, 1966; Rosevear, 1969; Harrison, 1972), or primarily nocturnal (Schmutterer, 1969; Ghobrail and Hodeib, 1982).

Laboratory evaluation of general activity rhythms of *A. niloticus* from a population 13°N of the equator revealed a somewhat diurnal pattern, with activity rising after the light phase began to a broad peak at midday, and falling again before the dark phase began (Duplantier and Granjon, 1990). However, the absolute peak level of activity occurred during the dark phase, due to another rise in activity that began shortly before the dark phase began, and peaked two hours later. Totals of hours during which activity occurred showed *A. niloticus* to be equally active in the dark and light phases. The authors did not report data from individual animals (they only reported mean values for several individuals), therefore it is unknown whether this was a general pattern, or whether the outcome reported was skewed by the rhythms of a few individuals.

Since these earlier reports from the literature describing the activity patterns of *A. niloticus* were conflicting, an analysis of their activity was undertaken in the present study (see Chapter IV).

I.4.e Demography and Social Organization

A. niloticus live in social groups called colonies (Delany and Happold, 1979). All colony members share a burrow system and a common foraging area (Packer, 1983). It is currently unknown how animals distribute themselves when they are underground, inside their burrows. Colonies contain approximately equal numbers of males and females (Delany and Monro, 1985b; Senzota, 1990). In Packer's two year study of a single colony in Tanzania, colony composition averaged 2.6 adult females, 3.1 adult males, and 4.9 immature individuals old enough to emerge from burrows. Senzota (1990) observed that animals of different age and sex categories in Tanzania seemed to associate with each other at random. He further noted that adults did not seem to defend their offspring against predators.

Packer (1983) found that male *A. niloticus* in Tanzania changed colonies with significantly greater frequency than did females. Thus, the colony consisted of female kin and their young, together with adult males originating from elsewhere. Females apparently may disperse to form new colonies, but do not join existing ones (Packer, 1983). Senzota (1990) found that there was a greater tendency for males than females in Tanzania to move from one colony to another, but that this tendency was not statistically significant. He also found that the number of colonies founded by males and females did not differ significantly.

The observations of Delany and Monro (1985b) suggested the occurrence of more movements between colonies than was indicated by Packer's (1983) data. Delany and Monro (1985b) stated that animals of both sexes at their Kenyan study site explored new habitats, and this included emigration to distant existing colonies. Such movements were seen throughout most of the year (Delany and Monro 1985b). This finding was contrary to what was observed at Packer's (1983) site in Tanzania, where immigration appeared to be sexually dimorphic and seasonal, peaking between July and September.

Delany and Monro (1985b) reported home ranges for *A. niloticus* living along field edges in Kenya, measured as the length of the line over which they were trapped. In October, home ranges were 86 m for males and 47 m for females. Home ranges measured from February through April, when animal densities were high, were much smaller (males: 37 m, females: 38 m). Müller (1977) measured home ranges of *Arvicanthis* in Ethiopia using a grid of traps. Home ranges during the rainy season for males were 2750 m^2, and for females and juveniles were 950 m^2. In the dry season, during which there were higher densities of animals, home ranges were 1400 m^2 for males and 600 m^2 for females and juveniles. From the estimations of Delany and Monro (1985b) and Müller (1977), it appears that the home ranges of female and juvenile *Arvicanthis* are smaller than those of males, and that home-range size decreases with increased animal density.

One should note that the home-range sizes mentioned above are much larger than the foraging radius values of 6-7 meters reported by Packer (1983) and Senzota (1983). The observations made by these two investigators were concentrated around the burrow systems. They did not use trap lines or grids in efforts to estimate range sizes.

I.4.f Burrow Configurations

Vesey-Fitzgerald (1966) reported that *A. niloticus* in the Rukwa Valley, on the border between Zambia and Tanzania, dig their own burrows. Delany and Neal (1966) also saw evidence of animals digging their own burrows in Uganda. Poulet and Poupon (1978) noted that *Arvicanthis* modify preexisting cavities and crevices in the soil. Vesey-Fitzgerald (1966) reported that the burrow holes appeared to occur in pairs: one entrance with one exit. In taking a census of burrow systems, he found that larger groups of holes yielded no more animals than did smaller ones, and proposed that large burrows were indicative of longer use, not larger colonies. Delany and Neal (1966) reported that burrows of animals in Uganda were usually 20-70 cm deep, and Poulet and Poupon (1978) excavated a burrow that was 30 cm deep. The burrow had multiple entrances that led to a central chamber. In addition to the burrows, Delany and Neal (1966) noted that the animals also constructed surface nests in tussocks of grass, but did not report the circumstances under which these nests were used.

Arvicanthis construct their burrow systems at the bases of bushes, trees, rock piles, banks, trash heaps, and termitaria (Delany and Neal, 1966; Packer, 1983; Senzota, 1982). Roots of trees and bushes loosen the soil and make it easier for the rats to construct their burrows, while the canopy cover prevents rain from flooding the burrows (Senzota, 1982), and sufficient sunlight filters through to allow grass to become established (Coe, 1972).

The animals maintain runways through the grass, and use these for travel around the home range (Vesey-Fitzgerald, 1966). The rats voluntarily cut plant material and remove small objects in their paths. This behavior has been observed even on ground

lacking vegetation (Senzota, 1990). Delany and Neal (1966) found that, when the grass was high, such as during the rainy season, these runways appeared as tunnels through the vegetation. Runways were longer, more visible, and more numerous during the dry season (Senzota, 1990). Senzota (1990) proposed that the evolution of runway construction may have been driven partly by the need to escape predators. When the rats sense danger, they run along a runway to the nearest burrow. Packer (1983) observed that the only defense mechanism *A. niloticus* appeared to exhibit against predators was immediate flight into a burrow.

I.5 REPRODUCTIVE BIOLOGY

We may be able to predict some aspects of the reproductive biology of *A. niloticus* by looking to animals that are closely related taxonomically, or to animals that occupy similar ecological niches. In general, murid rodents are spontaneous ovulators, and are polyestrous. Although they may breed continuously in the lab, murids are usually seasonal breeders in the wild (Asdell, 1964). The estrus cycle length can range from 4-11 days, and gestation from 17-47 days. Most species of murids exhibit a post-partum estrus, although this is not universal, and delayed implantation and prolongation of gestation are common during lactation. If a female does not become pregnant as a result of post-partum copulations, cycling is usually interrupted nonetheless by lactational anestrous (Asdell, 1964; Hayssen *et al*, 1993).

Ecologically, *A. niloticus* appear to resemble the microtine rodents, in particular those of the genus *Microtus*. Microtine rodents are members of the rodent Suborder Myomorpha, but are usually assigned to Subfamily Microtinae, separate from the murids.

Members of both *Arvicanthis* and *Microtus* are small-bodied and herbivorous, and are important prey species in their habitats. Members of the genus *Microtus* have been described as diurnal, nocturnal and crepuscular, exhibiting seasonal shifts in the timing of activity in some species (Madison, 1985). I have already made comparisons between the two groups with regard to body conformation and population cyclicity. Among the various species of *Microtus*, there is much variability with respect to reproduction. Most species are induced ovulators, but at least one species is a spontaneous ovulator (Seabloom, 1985). Length and timing of the breeding season are variable, especially among voles found in areas of low snow cover during the winter. Environmental factors which may influence the seasonality of breeding in voles are photoperiod, temperature, water availability, and nutrition. Gestation duration among *Microtus* is generally 21 days (Keller, 1985), and there is usually a post-partum estrus (Seabloom, 1985).

I.5.a Seasonality of Reproduction

Short-lived animals must reproduce as frequently and continuously as possible to offset their brief life span. Whether an animal reproduces seasonally or continuously depends upon the environment in which it lives (Bronson, 1989). Environmental factors known to influence the seasonality of reproduction in mammals include food availability, social cues, photoperiod, temperature, humidity, and rainfall (Bronson, 1987). Many mammals living below 30° of latitude appear to breed opportunistically in relation to food quality and availability, and thus often in relation to rainfall patterns (Bronson, 1989).

Delany (1986) described *A. niloticus* as highly adaptable, breeding both seasonally and aseasonally, depending upon the location of the population and upon local

environmental conditions. Neal's (1981) interpretation was that *A. niloticus* are both physiologically and ecologically well adapted for continuous breeding. Where *A. niloticus* breed aseasonally or continuously, litter sizes are small, but litters are larger in areas where there is a distinct breeding season (Delany, 1986). In describing the seasonality of reproduction in this species, Neal (1981) suggested that we should ask, not what initiates breeding, but what prevents it from occurring continuously?

Reproductive activity in tropical African rodents has been related to rainfall by some previous workers (Delany, 1972; Taylor and Green, 1976; Delany and Happold, 1979). Proposed mechanisms underlying a reproductive response to rainfall include: increased water intake (Neal, 1981), increased quantity and quality of food (Delany and Happold, 1979), and stimulation from chemical signals in the vegetation (Negus and Berger, 1977). A secondary plant compound found in green vegetation, 6-methoxybenzoxazolinone (6-MBOA) stimulates reproductive activity in the meadow vole, *Microtus montanus* (Berger *et al*, 1981); and since 6-MBOA has a stimulatory effect on the ovaries of another tropical murid rodent (*Mastomys coucha*) (Linn, 1991), the chemical may affect *Arvicanthis* as well. Kingdon (1974) cites a study in which *Arvicanthis* were induced to breed in the laboratory by supplementing the diet with fresh greens, and providing nesting boxes (Weinbren and Mason, 1957). However, among *A. niloticus* in the wild, the relationship between breeding and rainfall is variable (see Table 2 and Figure 7). In eastern Africa (Figure 7: A, B, C, D, E) breeding commences (or peaks, in the case of Packer's (1983) work) in concert with, or shortly after, a rise in rainfall. In Nigeria (F), however, breeding commences well before the onset of the rains. Further west, in Burkina Faso and Mali (G, H), the breeding season occurs completely

Table 2. Relationship between rainy season and reproductive season in nine African *Arvicanthis* populations. Note: Breeding seasons were adjusted for Taylor and Green (1976) and Neal (1981), in which the authors reported the presence of pregnant females. The authors' breeding season estimations were adjusted for the gestation of the pregnant females. All other authors were assumed to have made this adjustment in their estimation of breeding season, as they indicated that breeding season was derived mainly by noting the presence of pregnant or lactating females.

Investigator	Latitude	Study site	Rainy season	Reproductive season
Packer (1983)	02 20 S	Serengeti Natl. Park, Tanzania	March-May (peak)	March-May (peak)
Ghobrail and Hodeib (1982)	?	Sudan	July-September	August-February
Delany (1964b)	Equatorial	Queen Elizabeth Park, Uganda	April-May September-November	Only pregnant female was found in September
Delany and Roberts (1976)	00 16 S	Nakuru, Kenya (Rift Valley)	February-November March-April (peak)	Most intense in middle of the wet season
Rabiu and Fisher (1989)	12 03 N	Kano, Nigeria	May-September	March-November (1984) April-October (1985)
Taylor and Green (1976)	00 16 S	Kitale, Kenya	April-mid-December April and August (peaks)	May-December
Delany and Monro (1986)	00 16 S	Nakuru, Kenya (Rift Valley)	March-September	April-February
Neal (1981)	00 11 S	Mweya Peninsula, Uganda	March-May,	February-July,
	00 06 S	Crater Track, Uganda (Queen Elizabeth Park)	September-November	August-September
	00 11 N	Rojewero Plains, Kenya	March-May, October-December	February-December
Sicard *et al.* (1992)	14 00 N 13 00 N	Burkina Faso Mali	June-September May-October	November-April, September-June

Figure 7. The relationship between rainfall, temperature, and breeding season in *Arvicanthis.*

Breeding season is represented by the cross-hatched boxes, rainfall by the open circles (○), and temperature by the closed circles (●).
A. Kitale, Kenya (Taylor and Green, 1976), B. Nakuru, Kenya (Delany and Monro, 1985), C. Meru, Kenya (Neal, 1981), D. Queen Elizabeth Park, Uganda (Neal, 1981), E. Serengeti National Park, Tanzania (Packer, 1983), F. Kano, Nigeria (Rabiu and Fisher, 1989), G. Burkina Faso (Sicard *et al*, 1992), H. Mali (Sicard *et al*, 1992)

For graphs A-F, temperature and rainfall data were collected from *Agroclimatological Data for Africa* (1984). If a listing was not found for an exact location, the nearest location with a similar elevation was used (graphs C-E). For graphs G and H, temperature and rainfall data supplied in Sicard *et al.* (1992) were used.

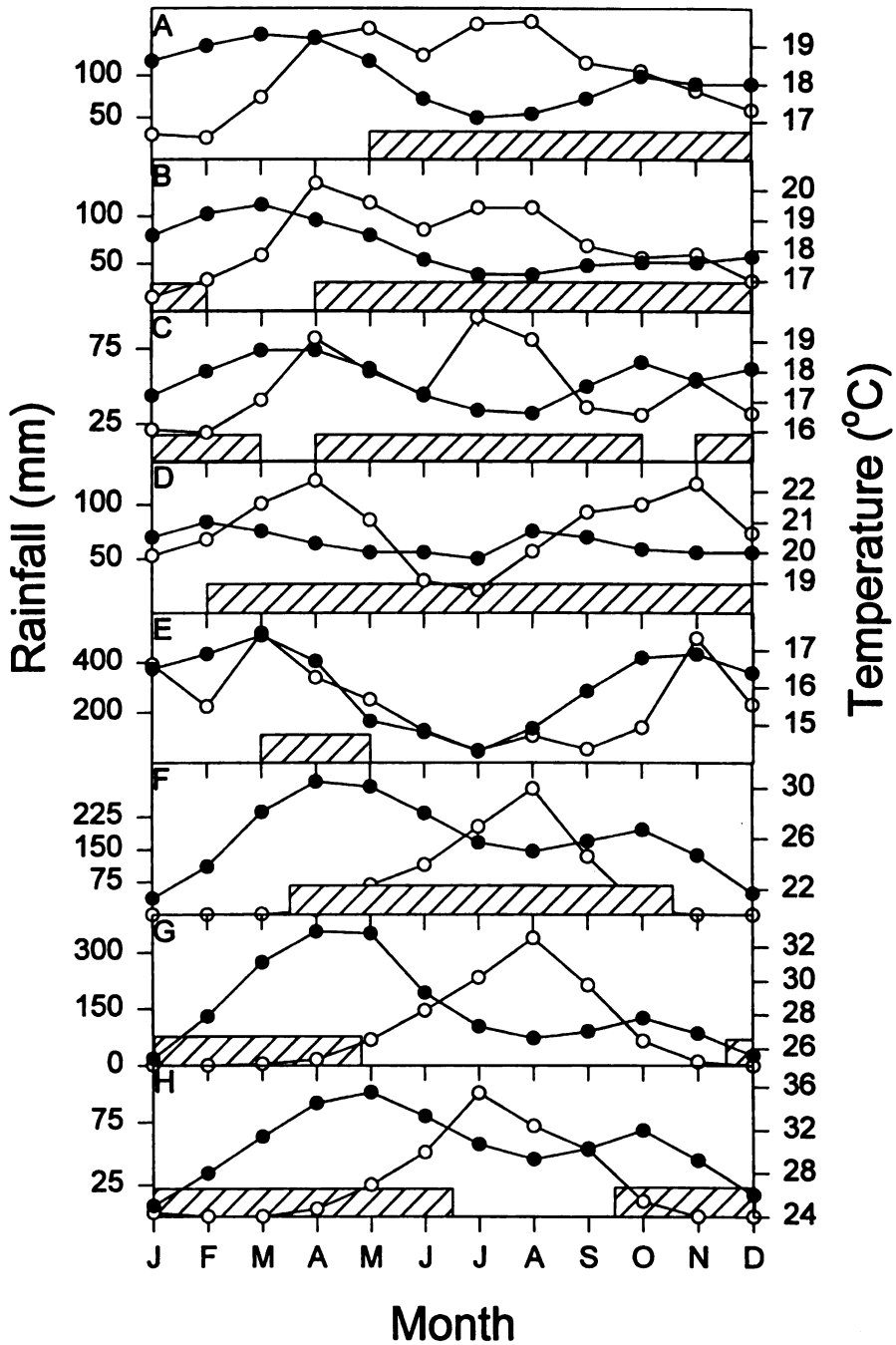


Figure 7.

opposite of the rainy season. Thus, although a correlation can be seen in eastern Africa, rainfall is not a good predictor of breeding season in the genus *Arvicanthis* across the continent.

Eastern Africa

Reproduction in *A. niloticus* in eastern Africa appears to occur most commonly during the rainy season (Delany and Roberts, 1976; Packer, 1983; Delany and Monro, 1986). Taylor and Green (1976) proposed that the breeding season of *A. niloticus* is controlled by diet, with abundance of necessary foods mediated by rainfall. They found that *A. niloticus* in Kitale, Kenya began breeding 2-3 months after the rains began, at the time when weed seeds were first found in stomach contents. Breeding continued throughout the rainy season and into the early part of the dry season, when it began to decline. As breeding declined, weed seeds and cereals were still present in stomach contents, but were less abundant. Later in the dry season, when breeding ceased, the diet of *A. niloticus* switched first to the leaves and stems of dicotyledonous plants, and then almost exclusively to grass. Fat deposits that were laid down while the more fatty seeds and cereals were abundant were rapidly utilized at this time. Fat stores were often completely depleted by the time breeding resumed. Taylor and Green (1976) proposed a relationship between reproduction and diet, suggesting that breeding occurred when seeds made up a major portion of the diet, and ceased when the diet switched to green vegetation.

Taylor and Green (1976) tested their hypothesis that the breeding season of *A. niloticus* is dependent upon food quality by provisioning animals with wheat for an entire year. The provisioned animals bred continuously, while unprovisioned controls

continued to show seasonality of breeding. It is clear, therefore, that the role of diet in regulating breeding of *Arvicanthis* in Kenya should not be discounted entirely. This finding is important with regard to the population outbreak of *Arvicanthis* in Kenya described by Taylor (1968). In years in which the rainy season is prolonged, the duration of cereal production may also be lengthened (Poulet and Poupon, 1978; Sicard *et al*, 1994). Since *A. niloticus* in east Africa appear to be more likely to breed when cereals are present in the diet, prolonged production of cereals may lead to an extended breeding season. An extended breeding season would result in higher than normal population numbers, or population outbreaks.

Neal (1981) argued that the results from Taylor and Green's (1976) study did not indicate a breeding season defined by food quality. First, they noted that breeding declined before nutritious food disappeared from the diet. Taylor and Green (1976) explained this as "allowing resources from the last of the season's weed seeds and cereals to be diverted from reproduction to fat" (p. 373). This does not, however, indicate a reliable cue for the inhibition of reproduction. Neal (1981) claimed that cessation of breeding may actually have been due to increased density of rodents as a result of decreased habitat due to harvesting in the study area. Second, in the second year of their study, reproductive activity began before seeds or cereals were detected in the diet. The commencement in activity was correlated with increased rainfall. Third, in Neal's (1981) observations in Kenya, there was no correlation between breeding and diet, and reproductive activity began a few days after the rains began, long before seeds and cereals were available.

Neal (1981) stressed the tie between dietary conditions and rainfall in governing timing of reproduction in *Arvicanthis*. He pointed out that breeding in Taylor and Green's (1976) study declined before seeds became unavailable, and that therefore seeds in the diet were not a reliable inhibitive cue. However, female mammals need to time their reproductive activity such that optimal resources are available at the point in their cycle when they need them most, usually during late lactation (Bronson, 1989). A female *Arvicanthis* would need to 'know' how long seeds would be available, not just whether they were available. So, while the presence or absence of seeds in the diet might not be the proximate cue causing induction and cessation of reproduction, it might often be the ultimate factor responsible. This hypothesis is consistent with the results of Neal's (1981) study. Perhaps Neal's (1981) animals started breeding right after the rains began because they used rain as a proximate cue for the ultimate factor of improved dietary conditions.

Neal (1981) failed to find a relationship between diet and reproduction in Uganda and Kenya, where breeding seemed to be controlled by temperature. He concluded that breeding was most likely inhibited by water stress due to high temperatures, and that this would explain the increase in breeding seen when the rains began. This is a point well taken, for even a small increase in ambient temperature above a mammals' thermoneutral zone can increase demand for water, and decrease food intake and locomotor activity (Bronson, 1989), creating suboptimal conditions for breeding.

In a comparison of animals from Uganda and Kenya, Neal (1981) found that female reproductive activity and pregnancy were significantly higher during the wet seasons in both places. Delany (1964b) examined 38 female *Arvicanthis* collected from

July to September in Queen Elizabeth Park, Uganda, for signs of reproductive activity. Of these 38 animals, only one was pregnant and one lactating. The pregnant female was found in September, 1963 while the rest were collected in July and August. According to Delany (1964b) there is a lull in rainfall from June through August. It is possible that Delany found such a low rate of reproductive activity due to the fact that he was collecting animals during a time of low rainfall. In an earlier collection period in 1961 from July through December in Queen Elizabeth Park (Ankole and Karamoja), the author found only one pregnant female (in August) out of 15 collected (Delany, 1964a). This is in sharp contrast to Delany and Neal's (1969) 1965-1966 studies in Queen Elizabeth National Park, in which *Arvicanthis* bred continuously, and in which breeding peaked in October, a month of high rainfall (these data appear to be the same as those Neal reported in his 1981 paper). It is possible that, if *Arvicanthis* are using rainfall as a proximal cue of eventual food availability, they might be able to change their breeding season with changing rainfall patterns.

Western Africa

Rabiu and Fisher (1989) found that *Arvicanthis* in Nigeria began to breed 1-2 months before the rains, and stopped breeding at the end of the rainy season. The authors describe 3 possible reasons or cues for this early commencement of reproduction: a rise in ambient temperature in March and April, a fall in fat reserves towards the end of the dry season, or a change in photoperiod, but these possible cues were not examined experimentally. It is quite possible that photoperiod was the cue that triggered the commencement of breeding. The effect of photoperiod on the breeding season of *Arvicanthis* in western Africa is described below.

While Bronson (1989) stated that it is unlikely that animals living below 30° of latitude respond to changes in photoperiod, he maintained that the capacity to respond reproductively to photoperiodic cueing remains as a neural trait, depending upon the animals' evolutionary history. This assertion was mainly in response to a group of investigators who studied *Arvicanthis* living further away from the equator than the studies mentioned above (Sicard *et al*, 1988). This group of investigators considered the possibility that photoperiod may be governing the seasonality of reproduction of *Arvicanthis* in western Africa (Sicard *et al*, 1992, 1993, 1994). In years of normal rainfall, the breeding season of *A. niloticus* in Burkina Faso and Mali in west Africa does not overlap with the rainy season. Other investigators in Ethiopia and Sudan have also found that *Arvicanthis* are capable of breeding during the dry season (Müller, 1977; Ghobrail and Hodeib, 1982). Sicard *et al.* (1988) tested the effect of photoperiod on testicular function in sexually active male *A. niloticus* from Burkina Faso. Even though the range of daylength in Burkina Faso is small (11 h 09 min-12 h 52 min), manipulation of photoperiod had an effect on testosterone production in test animals, as compared to controls maintained in a natural photoperiod. Interestingly, the effect was bimodal, there was a photostimulatory phase between 8-11 h of daylight, and a photoinhibitory phase when daylight exceeded 11 h 30 min.

Sicard *et al.* (1992) repeated this experiment using animals from both Burkina Faso and Mali. The range of daylength in Mali is similar to that in Burkina Faso (11 h 11 min-12 h 49 min). The investigators were able to replicate the findings of their 1988 paper, in that males from Burkina Faso displayed decreased testosterone production when exposed to a daylength exceeding 11 h 30 min. The animals from Mali showed decreased

testosterone production in daylengths exceeding 12 h 15 min, although decreases of testosterone in these animals did not reach the significance level of the data from the Burkina Faso animals. This experimental evidence is supported by field observations of the breeding seasons of animals in these two areas. Thus, having shown that a change in daylength affects reproduction of *Arvicanthis* in some parts of Africa, Sicard *et al.* (1988, 1992) successfully responded to Neal's (1981) appeal that the factors inhibiting reproduction, rather than the factors facilitating reproduction in *A. niloticus* be investigated.

While it may seem that a reproductive season governed by photoperiod would leave little room for variation in length of breeding season, I have noted that it is a lengthening of the breeding season of *A. niloticus* that is cited as facilitating the outbreak of these rodents in Burkina Faso in 1986 (Sicard *et al*, 1994). In order to determine whether photoperiodic regulation of the reproductive season could be modified by other factors, Sicard *et al.* (1993) investigated the effects of temperature and water conditions (relative humidity and availability of water rich foods) on testicular function in male *A. niloticus* from Burkina Faso and Mali. Animals from Mali were tested in a short photoperiod, while those from Burkina Faso were tested in both short and long photoperiods. Testosterone levels increased in sexually inactive animals housed in long daylengths but at low temperature and with abundant water. This increase was most noticeable in animals housed in a short photoperiod.

To summarize, the onset and durations of breeding seasons of *Arvicanthis* near the equator, as evidenced in this review by animals in eastern Africa, may be influenced by food quality, water availability, or temperature. *Arvicanthis* living further from the

equator, like those in this review from Nigeria, Mali, and Burkina Faso, display breeding seasons that appear to be mediated by photoperiod, and modified by food quality, water availability, and temperature. The effects on breeding of social cues such as population density have not yet been formally studied in *Arvicanthis*.

I.5.b Estrous Cycle

There is only one account of the estrous cycle of *A. niloticus*. Using the vaginal smear technique, Compoint-Monmignaut (1968) described a 6 d estrous cycle for a single female in the laboratory. This author did not report sample sizes or housing conditions, but described all other females as being in permanent anestrus. While the author did not offer an explanation for the incidence of permanent anestrus, it may have occurred due to overcrowding, stress or poor husbandry. It is also possible that not all females cycle at all times, or that there were confounding seasonal or ontogenetic variables. Ghobrail and Hodeib (1982) took daily vaginal smears to elucidate the estrous cycle of this species. However they merely stated in their results that the smears seemed to be comparable to those of spontaneously ovulating rodents, and did not report a cycle length. As occurs in most murids (Bronson, 1989), female *A. niloticus* exhibit a post-partum estrus (Ghobrail and Hodeib, 1982; Delany and Monro, 1985a).

I.5.c Ovulation

Sicard *et al.* (1993) made reference to a thesis (Kyelem, 1993) that described *A. niloticus* as spontaneous ovulators that do not exhibit a seasonal anestrus. The only published study in which ovarian histology of *A. niloticus* has been examined to date was

that conducted by Ghobrail and Hodeib (1982), who collected animals over an 18 month period in Sudan. They found that their wild-caught adult female *A. niloticus* were continuous breeders, as indicated by the fact that their ovaries contained either large follicles or corpora lutea of ovulation, and that there were no reproductively quiescent animals. Corpora lutea were always present in the ovaries of pregnant and lactating females. The ovaries of juveniles contained primary and secondary follicles, whereas those of mature, non-pregnant adults contained follicles at all stages of development, including fully mature follicles. Ghobrail and Hodeib (1982) reported that corpora lutea were never seen in mature non-pregnant adults. This information, given in the text, is contradicted by measurements of corpora lutea in recently mature and mature, non-pregnant females presented in Ghobrail and Hodeib's (1982) Table 3 (p. 326). The authors did not report whether these females were nulliparous or multiparous. They found that mature females kept solitarily, in pairs with males, or in heterosexual groups showed corpora lutea of ovulation. The fact that the ovaries of solitarily housed females contained corpora lutea suggest that *A. niloticus* is probably a spontaneous ovulator. Since the exact conditions of the females in the above study were unknown, in the current study I examined the ovaries of a variety of age groups in order to determine whether *A. niloticus* are spontaneous or induced ovulators.

I.5.d Gestation

Previous reports of gestation length in *A. niloticus* have varied. Estimations for gestation length among females not suckling a litter are as follows: 18 d (Delany and Happold, 1979), 21-23 d (Ghobrail and Hodieb, 1982), 22-24 d (Delany and Monro,

Table 3. *Arvicanthis* litter size in captivity and in the field.

Investigator	Study site	Average litter size	Range
Packer (1983)	Tanzania above-ground observations	3.7	1-7
Rabiu and Fisher (1989)	Nigeria burrow excavations	5.4	not given
Quilici *et al.* (1969)	lab stock from Senegal	not given	5-10
Delany and Monro (1985a)	lab stock from Kenya	3.7	1-6
Ghobrail and Hodeib (1982)	Sudan lab stock: combined pup and embryo counts	November-March: 5.4 April-August: 3.5	4-12
Taylor and Green (1976)	Kenya capture data: embryos	6.0	2-12
Coe (1972)	Kenya capture data: embryos	1968: 3.0 1970: 6.5	not given
Delany and Happold (1979)	not given	4.0	3-10
Rabiu and Fisher (1989)	Nigeria capture data: embryos	7.3	not given
K. E. Holekamp (personal communication)	Kenya capture data: pups born to females in captivity that were pregnant when captured	1992-1993: 4	3-5

1985), or 24-26 d (Quilici et al. 1969). Ghobrail and Hodieb (1982) also reported a gestation of 35-51 d for females suckling litters. Since these earlier estimations were inconsistent, a study of gestation length in *A. niloticus* was undertaken in this thesis.

I.5.e Litter Size

Bronson (1989) stated that the reproductive strategies of murid rodents are driven by the need to compensate for a very short life expectancy. One of the ways in which murids make up for a short life span is to produce large litters at a high rate. Listed in Table 3 are litter sizes for *Arvicanthis* from direct observation of litters in the wild, from lab data, and from capture data. Please note that most of the capture data are reported in counts of embryos, not of pups. Embryo counts of up to 10 (Delany and Happold, 1979) and 12 (Taylor and Green, 1976) indicate that *Arvicanthis* has the capacity to produce large litters. However, as the female has only six mammae, it is not likely that litters of this size would be seen in the wild. This is also reflected by the fact that the average litter sizes reported by Packer (1983) and Rabiu and Fisher (1989), were 3.7 and 5.4, respectively. These averages are smaller than the reports of embryo counts mentioned above. Packer's (1983) range of 1-7 from observations of litters in Tanzania does not approach the ranges of 5-10 (Quilici *et al*, 1969) and 2-12 (Taylor and Green, 1976) given for the laboratory. We have had litters of 12 pups born in our M.S.U. lab colony as well.

I.5.f Lactation Interval

Delany and Monro (1985a) noted evidence of lactation in mothers for three weeks after parturition. These authors proposed that since a second litter could be born at this

time, weaning probably took place during the third week post-partum.

I.5.g Growth and Development

Delany and Monro (1985a) charted growth of *A. niloticus* in the laboratory and in the field. Neonatal mass in the lab (4.65 ± 0.18 g) and in the field (4.25) were similar, but laboratory animals exhibited considerably faster growth rates. Delany and Monro's (1985a) study of development in the lab revealed that *A. niloticus* were born with short, dark fur, and that their coats took on the speckled coloration of adults within one week. Incisor teeth in lab-reared pups erupted at 3 days. These pups opened their eyes at 7 days, and had full motor ability by 14 days (Delany and Monro, 1985a). Quilici *et al.* (1969) found that eyes were open and incisors apparent in lab-bred pups at five days, which is closer to what was seen in our M.S.U. lab during this study. Delany and Monro (1985a) found that weaning occurred in lab-born pups when they weighed 25 grams, which is similar to Neal's (1981) field estimate of 15-25 g.

Measurements of *A. niloticus* from our colony are listed in Table 4 (n=45 females, 15 males). In general, nulliparous females in our colony are smaller than males, but pregnant or lactating females are often difficult to differentiate from males in terms of body mass.

Table 4. Mean measurements (± SE, [range]) of 45 female and 15 male *A. niloticus* from the M.S.U. colony.

Sex	Weight	Hind foot	Ear	Tail	Head and Body	Total Body
Male	103.5 ± 6.7 (55-103)	27.3 ± 0.5 (25-30)	16.6 ± 2.5 (11-20)	87.4 ± 1.8 (75-100)	137.9 ± 3.2 (111-156)	225.3 ± 3.8 (186-243)
Female	84.6 ± 3.1 (46-120)	27.3 ± 0.5 (21-35)	15.5 ± 0.3 (10-20)	87.3 ± 1.1 (68-104)	132 ± 2.7 (101-204)	219.2 ± 2.9 (187-280)

I.5.h Ages for Males and Females at Sexual Maturity

Age at sexual maturity probably depends upon environmental conditions. Neal (1981) found that animals from Uganda and Kenya matured at different rates. Quilici *et al.* (1969) found that *A. niloticus* in the lab were capable of breeding at 40-50 d, although females matured slightly later than males. Delany and Monro (1985a) also noted scrotal testes in lab-raised males at 40-50 days. First parturition in females can occur at 60 d (Delany and Monro, 1985a). Given approximately 25 d of gestation, these data on age of first parturition suggest that puberty in females occurs at approximately 45 d.

Reproductive maturity in wild populations is usually related to body mass, since exact ages are frequently unknown. Neal (1981) found that puberty occurred at very different weights in animals in Uganda (males: 65-80 g; females: 55-65 g) and Kenya (males: 33-40; females: 20-35), but this was probably due to a similar difference found in the weights of adults in these two countries (Uganda males: 70-125, females: 60-110; Kenya males: 60-90, females: 60-90). Taylor and Green (1976) reported that the weight at which puberty occurred in free-living *A. niloticus* in Kenya was 45 g, based on the lowest weight recorded for a pregnant female.

I.5.i Life span

Murid rodents usually live less than two years, and frequently live less than one year (Walker, 1975). Females in Packer's (1983) study in Tanzania lived an average of 10.2 months (maximum: 20 months), but the life span of males was more difficult to quantify because of dispersal. Neal (1981) noted that few animals in Uganda and Kenya lived as long as a year, and Delany and Monro (1986) found that their animals in Kenya had a five percent chance of living to one year of age. Müller (1977) estimated that the life expectancy for a one month old animal in his Ethiopia study was seven to eight months. Thus in the field, the life span of *Arvicanthis* is less than one year, and it is unknown at this point whether there is a sex difference in longevity.

Animals living in captivity, with the luxuries of *ad libitum* food and protection from predators, often live longer than their counterparts in the wild. There is one record of a captive *Arvicanthis* living for 6 years and 8 months (Jones, 1982, cited in Nowak, 1985).

CHAPTER II

THE NON-PREGNANT ESTROUS CYCLE AND OVULATION

II.1 INTRODUCTION

Most female mammals will accept copulation with males only at times when they are physiologically ready to do so, and these short periods of sexual receptivity are known as heat, or estrus. Periods of estrus are separated by longer periods of time in which the female is not physiologically ready to mate, and in which she is unreceptive to male advances. These periods of estrus and non-estrus may occur repeatedly and sequentially, and they constitute the estrous cycle (Sadleir, 1973).

The estrous cycle has been studied in great detail in laboratory and domestic species of rodents, such as *Rattus norvegicus* (Long and Evans, 1922), *Mesocricetus auratus* (Kent and Smith, 1945), *Cavia porcellus* (Stockard and Papanicolaou, 1917), and *Mus musculus* (Allen, 1922). While this work has been invaluable to the study of reproduction, it does not reflect the diversity of the Order Rodentia (Hayssen *et al*, 1993). The species mentioned above are highly inbred, specialized forms that are the result of years of domestication and artificial selection, and the information gained from studying these animals may have little bearing on what actually occurs in wild animals (Conaway, 1971). It has also been suggested that the domestication process for *R. norvegicus* has selected for short ovarian cycles, as well as successful mating abilities under laboratory

conditions (McClintock and Adler, 1978).

Historically, reproductive cycles in female mammals have been classified into one of two categories: those in which ovulation occurs spontaneously, and those in which ovulation is induced by some form of stimulation (e.g. Eckstein, 1949). As our knowledge of the field has grown, this dichotomy has proven not only inadequate, but constraining. We have been eager to put a species or genus into one category or the other, missing out on important subtleties essential to our knowledge of reproduction as a whole. Conaway (1971) broadened the traditional dichotomy into a spectrum, noting that spontaneous and induced ovulation are the extremes of a single continuum, and both patterns of ovulation are capable of being modified by various factors. Intensive study of domestic rodent species has led to an oversimplified conceptualization of mammalian reproduction, and often to the belief that spontaneous ovulation is the rule, with induced ovulation being the exception. In fact, as more information is obtained regarding the reproductive cycles of various mammalian species, it seems more likely that induced ovulation is the basic pattern, and spontaneous ovulation a specialization (Conaway, 1971).

In light of the common contemporary belief that spontaneous ovulation is the rule among mammals, it is surprising that induced ovulation was thought to be the predominant mammalian strategy until the beginning of the 19th century. It wasn't until corpora lutea were found in the ovaries of virgin women that the idea of spontaneous ovulation became popular (Ramirez and Soufi, 1994). The fact that human females are spontaneous ovulators has perhaps led to an anthropocentric view of ovulation. However we are learning that, as is often found in mammalian reproduction, many different means

exist to achieve the same end.

Conaway (1971) expanded the traditional spontaneous/induced categorization of non-pregnant female reproductive cycles into three categories, with category assignments made on the basis of spontaneous vs. induced forms of ovulation, and luteal function (Table 5).

Table 5. Categorization of non-pregnant mammalian female reproductive cycles (after Conaway, 1971).

Type	Ovulation	Luteal Function
Type I Subtype A: medium length cycles. Subtype B: long cycles	spontaneous	spontaneous
Type II Subtype A: medium length cycles. Subtype B: long cycles.	Induced	spontaneous
Type III	spontaneous	induced

In Type I, both ovulation and pseudopregnancy occur spontaneously. This condition is typical of the hystricomorph rodents, ungulates, and primates. *Cavia porcellus*, the guinea pig, frequently used in laboratory studies, displays an estrous cycle of 15-18 days (Subtype IA). This is significantly longer than the cycle seen in the rat and the hamster, because the corpora lutea in *C. porcellus* are functional in the absence of copulation (Feder, 1981). The follicular phase is short, and the luteal phase lasts 12-13 days (van Tienhoven, 1983). Canids are classified in Subtype IB, with non-pregnant

cycles that are over five weeks in length (Conaway, 1971).

Animals categorized as Type II are induced ovulators, with spontaneous corpus luteum formation. Ovulation is induced by exogenous stimuli, most notably copulation. This strategy is employed by several species of rodents in the genus *Microtus*. *Microtus* are placed in Subtype IIA, with medium length cycles. In *M. ochragaster*, both behavioral estrus and ovulation are induced, and if stimulation is continuous, behavioral estrus can last for at least 30 days (Richmond and Conaway, 1969). Felids, which are in Subtype IIB, come into behavioral estrus spontaneously, and the estrus period is more fixed. Non-pregnant cycles in this subtype last 4-8 weeks (Conaway, 1971).

In animals that are classified as Type III, ovulation is spontaneous, but luteal function is contingent upon copulatory stimulation. *Rattus* and *Mesocricetus* fall into the Type III category. The cycle is short, 4 days for hamsters and 4-5 days for rats (Conaway, 1971).

Milligan (1975) suggested the addition of a fourth type, in which both ovulation and luteal function are induced. In the field vole (*Microtus agrestis*), ovulation may be induced by stimulation from a male, yet the stimulation may not be sufficient to induce luteal function. That is, ovulation and luteal function appear to have different stimulation thresholds for activation.

As I stated in Chapter I, little is known about the reproductive cycle of *A. niloticus*. Ovulation in wild-caught females was thought to be spontaneous by Ghobrail and Hodeib (1982), and a 6 d estrous cycle was described for a single female housed in captivity by Compoint-Monmignaut (1968). The purpose of the work presented in this chapter was to describe the non-pregnant estrous cycle and ovulatory pattern of *A.*

niloticus. I used a variety of methods in my attempts to elucidate the estrous cycle, including cytological assays of vaginal smears, behavioral assays, and long-term monitoring of activity rhythms. Ovarian histology was performed at the conclusion of most of these studies, in order to determine the pattern of ovulation in this species.

II.2 CYTOLOGICAL ASSAYS OF VAGINAL SMEARS

When Stockard and Papanicolaou reported the existence of a regular estrous cycle in the guinea pig in 1917, they established the validity of the vaginal cytological assay. The authors showed that changes in cell content of the vaginal lumen corresponded to changes over the ovarian cycle, thus providing a relatively noninvasive marker of ovarian activity. This assay has since been used to describe the estrous cycles of many mammalian species (Rowlands and Weir, 1984).

By comparing the relative density of the 3 types of vaginal cells (cornified epithelial cells, nucleated epithelial cells and leukocytes), one can assign each smear to one of the 4 phases of the estrous cycle. These are estrus, metestrus, diestrus and proestrus, and cytological changes in the vaginal lumen with the phase of the female cycle are depicted in Figure 8. Estrus is indicated by a smear that consists solely of cornified epithelial cells (Figure 8, C). A diestrus smear contains many leukocytes, and a few cornified epithelial cells (Figure 8, D). Metestrus is indicated by a vaginal smear that contains predominantly leukocytes (Figure 8, A). A proestrus smear contains mostly nucleated epithelial cells, occurring singly or in sheets (Figure 8, B).

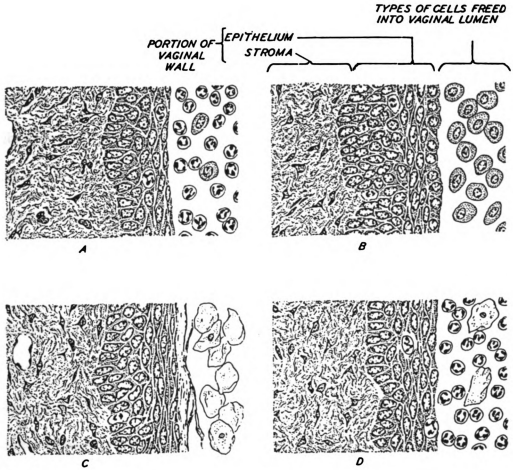


Figure 8. Cyclic changes in vaginal cell types.(after Turner and Bagnara, 1971).

II.2.a General Methods

Subject Animals and Housing Conditions

The original stock of 29 *A. niloticus* from which the Michigan State University breeding colony is derived was captured in July and August 1993, from two locations in the Masai Mara National Reserve in southwest Kenya. *A. niloticus* in the M.S.U. breeding colony were housed in plastic cages (38x14z16cm) with chipped aspen litter, and cages were changed once a week. Animal rooms were kept at 23°C on a 12:12 light:dark cycle. Animals were provided *ad libitum* tap water and Harlan 8640 Teklad 22/5 rodent chow, supplemented with carrots and whole oats once a week. Litters born to mating couples were weaned at 21 days, and the opposite-sex siblings were separated at 50 days, after which only littermates of the same sex were housed together.

Cytological Assay of Vaginal Smears.

Females were housed individually. In each experiment, vaginal smears were obtained from each study animal at 24 h intervals at the same time every day, for a series of consecutive days. Vaginal lavage was performed by releasing a few drops of 0.9% saline solution into the rat's vagina with a glass eyedropper. The saline was then drawn back into the eyedropper, and the sample was released and spread onto a microscope slide. The smears were air-dried overnight. The slides were then stained in 0.25% aqueous methylene blue, rinsed in distilled water, and stood on end to dry. The slides were viewed under a light microscope at 10x and 40x magnification, and the cell compositions of the slides were recorded. At the end of each experiment, the females were sacrificed, and their blood and reproductive tracts were stored for further analysis. Blood samples

were collected via cardiac puncture, plasma was separated by centrifugation, and kept frozen at -80°C. Ovaries and uteri were collected and stored individually in sealed bottles of 10% buffered formalin.

II.2.b Initial Evaluation Of Cell Types

Purpose

The purpose of this pilot study was to determine whether vaginal smears of *A. niloticus* contain the distinct cell types described in other rodents.

Methods

Vaginal smears were taken from two nulliparous females for 11 days and one multiparous female for 9 days.

Results

The three types of cells expected: leukocytes, cornified epithelial cells, and nucleated epithelial cells were readily visible in the vaginal smears of *A. niloticus* (Figure 9). Cell types changed in relative abundances in the smears of the virgin females, but estrous smears were never seen. The multiparous female gave a typical estrous smear every day.

II.2.c Females Housed In Small Cages

Purpose

The objective of this study was to systematically examine the vaginal smears of a large number of females in an effort to elucidate the estrous cycle of *A. niloticus,* and to compare multiparous females to nulliparous females with respect to estrous cyclicity.

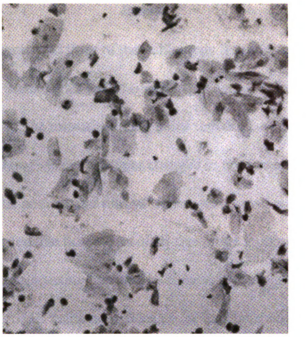


Figure 9. Vaginal smear from a nulliparous female *A. niloticus*.

Methods

Daily vaginal smears were taken from 5 multiparous females and 10 nulliparous females, between 1000 h and 1100 h for 29 days. Two of the multiparous females were of unknown age, and the other three were between 200-216 doa. Each of the multiparous females had delivered at least 2 litters. The nulliparous females were all at least 104 days old (range: 104-116 days).

Ovaries were fixed in paraffin (Appendix A), sliced on a rotary microtome, fixed onto microscope slides, and stained with hematoxylin and eosin (Appendix B). Ovarian sections were viewed under a light microscope at 10x and 40x magnification, and were scored for the presence of corpora lutea. Each section was scanned for luteal tissue, and a yes/no classification was recorded.

Results

All five multiparous and three of the nulliparous females gave estrous smears every day (Figure 10). Leukocytes would occasionally appear in an animal's smear, but in very low abundances. The remaining nulliparous females gave smears containing all three types of cells; although these cell types changed in relative abundances, they did not show the expected cyclic pattern, and estrous smears were never seen (Figure 11). Ovarian histology revealed that the ovaries of all five of the multiparous females contained corpora lutea, suggesting that all five females had ovulated. Luteal tissue was absent from the ovaries of all nulliparous females. A Student's t-test revealed that uterine weights, corrected for body weight, did not differ between multiparous and nulliparous females ($t = -1.15$, $df = 13$, $p = 0.272$).

II.2.d Females Housed In Large Enclosures

Purpose

I thought that perhaps the results from the first study did not follow the expected pattern of smear types due to the stress of captivity and of daily handling. Therefore the objective of this study was to modify the conditions of the previous experiment, in order to alleviate stresses on the animals.

Methods

I attempted to ease the stresses of captivity and handling by housing females individually 3.5 ft x 3.5 ft enclosures, and by anesthetizing the animals with Metofane prior to taking the samples. I took daily vaginal smears from four mature nulliparous females for 15 consecutive days. Having learned in a previous experiment (described in

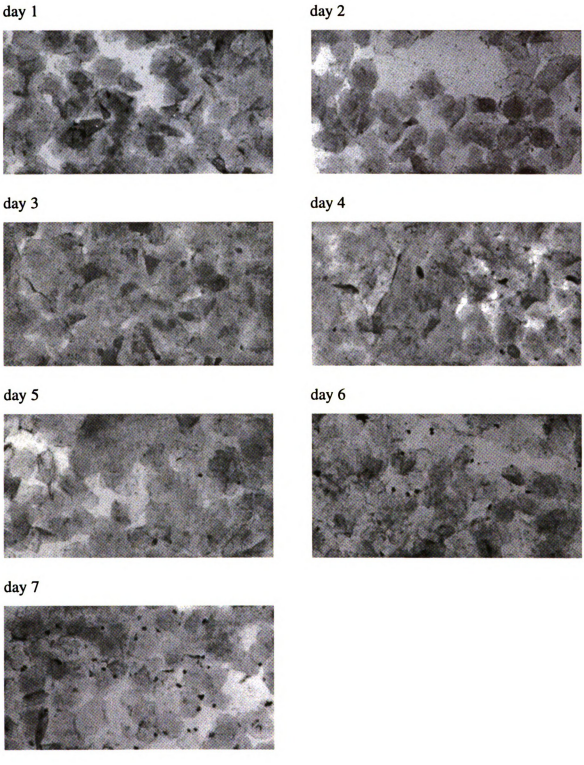


Figure 10. Persistent estrous vaginal smears from a nulliparous female *A.niloticus*.

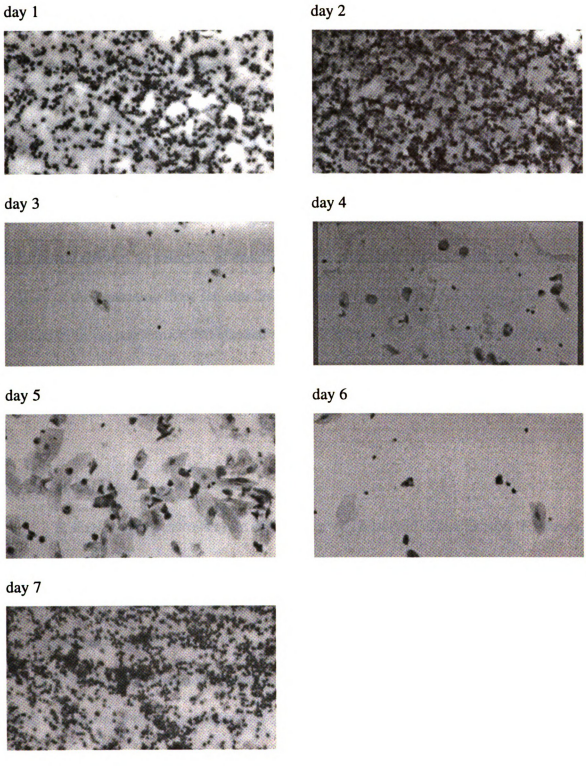


Figure 11. Erratic vaginal smear series from a nulliparous female *A. niloticus*.

Chapter IV) that copulations involving nulliparous female *A. niloticus* occurred around the time of the beginning of the light phase, I took the samples at 0700 h, just after the lights came on in the animal room.

Results

One female gave an estrous smear on the first day of the experiment, then proceeded to give smears that indicated a five-day cycle, and gave another estrous smear on the sixth day of the experiment (Figure 12). After this initial cycle, the smears from this female showed no evidence of cyclicity for the remainder of the experiment. The smears of the remaining three females did not give any indication of cyclicity. The ovaries from the one female that showed a vaginal estrous cycle contained no corpora lutea. Two of the remaining females similarly showed no evidence of ovulation, but the last female had one regressing corpus luteum in one of her ovaries.

II.2.e Conclusions

A single vaginal estrous cycle of five days was observed in one female. However, as the female that appeared to show a cycle did not show evidence of ovulation histologically, I conclude that this apparent vaginal cycle was not a reflection of an ovarian estrous cycle.

The pattern of continuous estrous smears shown by all multiparous females and three nulliparous females is puzzling. It is possible that this smear pattern may be the result an insufficient diet. Greenwald (1956) mentioned that a prolonged estrous smear in an isolated female rodent could conceivably be due to a vitamin A deficiency. In *R. norvegicus*, continuous estrous smears are indicative of aging, and of the cessation of

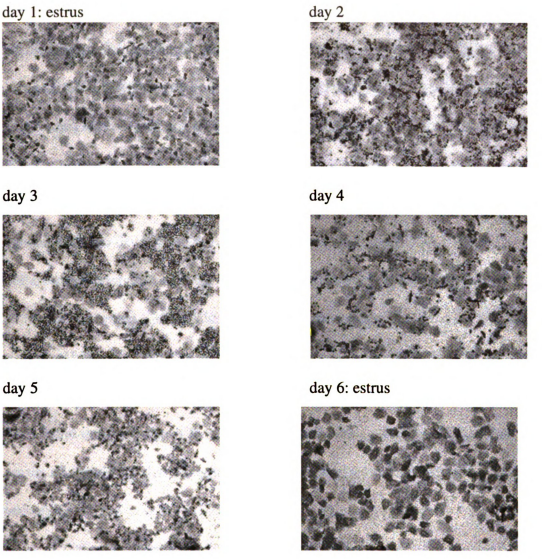


Figure 12. A five day estrous cycle from a nulliparous female *A. niloticus*.

estrous cyclicity. In one experiment, the ovaries of rats in constant estrus did not contain corpora lutea (Huang and Meites, 1975), indicating that ovulation had not occurred. The presence of corpora lutea in the ovaries of multiparous females indicates that constant estrous smears did not simply reflect aging in *A. niloticus* in this experiment.

Breed (1967) reported persistent estrous smears in the reflexively ovulating short-tailed vole (*Microtus agrestis*). yet corpora lutea were not visible in the ovaries of these females. Peterson (1986) found in the gray-tailed vole (*M. canicaudatus*) that the presence of persistent vaginal cornification was age-related, and that two-thirds of females 150-200 days of age showed persistent vaginal cornification. This is fairly early in the reproductive lives of these voles, since they will reproduce in the laboratory for 12-16 months (Peterson, 1986). Therefore, persistent vaginal cornification in the reflexively ovulating *Microtus* species is probably not analogous to the persistent estrous state associated with aging in the spontaneously ovulating *R. norvegicus*. Since corpora lutea were found in the ovaries of female *A. niloticus* that had exhibited persistent vaginal estrus, it is not likely that this smear pattern is analogous to constant estrus in the microtines.

Continuous estrus periods have also been observed in smears obtained from *Rhabdomys pumilio* (Dewsbury *et al*, 1984), a species closely related to *A. niloticus*. These periods were very similar to those seen in *A. niloticus*, with the occasional appearance of leukocytes on one day within the period of constant estrus. Dewsbury *et al.* (1984) found no obvious explanation for constant vaginal estrus, but mentioned that it may be advantageous for a female to be in a prolonged state of receptivity. Dewsbury *et al.* (1984) did not, however, comment specifically on the receptivity of the females

showing constant estrous smears. The authors merely stated that copulation was more likely to occur during proestrus, but that it may occur during estrus as well.

It is possible that continuous estrous smears in *A. niloticus* are due to high levels of circulating estrogens. Perhaps high hormone levels are 'masking' the vaginal representation of the estrous cycle. It is also possible that estrogen levels are normal for a murid rodent, but that this species has a lower threshold to estrogen stimulation for eliciting an estrous smear. Another possibility is that the stress of captivity and daily handling renders the vaginal cytological assay ineffectual in *A. niloticus*. Whatever the ultimate reason, it is clear that the cytological assay of vaginal smears was not an effective indicator of estrous cyclicity in our captive M.S.U. colony of *A. niloticus*.

The presence of corpora lutea in the ovaries of females isolated from all conspecifics for 30 days, suggests that *A. niloticus* can ovulate spontaneously. There is a remote possibility that this species is an induced ovulator, and that the females in this study were induced to ovulate by the stimulation of the glass eyedropper in the vagina during the smearing procedure. This seems unlikely, however, since luteal tissue is absent from the ovaries of virgin females. This finding may indicate that isolated virgin females do not ovulate. There are many possible reasons for this. Multiparous females may ovulate spontaneously, while nulliparous females may be induced to ovulate. That is, the female's first ovulation may be induced by the presence of a male, as occurs in the dwarf hamster (Erb *et al*, 1993). Multiparous females may actually be induced ovulators , but may have a lower threshold for ovulation to be induced, and may have been stimulated by the sampling process. It is also a possibility that the stress of handling suppresses ovulation in nulliparous females, but not multiparous females.

II.3 BEHAVIORAL ASSAYS

II.3.a Male-Female Interactions

The manner in which male and female mammals behave toward one another changes over the course of the female's estrous cycle. Sexual behavior of female mammals can be divided into three components: attractivity, proceptivity, receptivity (Beach, 1976). Attractivity is the female's stimulus value to male conspecifics, proceptivity is the extent to which the female initiates or solicits copulation, and receptivity reflects the stimulus value of a female for educing an intravaginal ejaculation from a male. These three behavioral parameters vary with circulating levels of steroid hormones produced by the ovary during the estrous cycle, and in many mammals estrogen enhances female sexual behaviors and progesterone inhibits them.

Purpose

The objective of this study was to elucidate the estrous cycle by observing the copulatory behavior of male-female pairs over a series of days. I hoped to observe cyclical changes in male-female interactions, reflecting cyclic changes in the female's reproductive physiology. My specific goal was to calculate lordosis quotients (LQs) in female *A. niloticus*, and to monitor changes in LQs over time. The LQ is the ratio of the number of lordosis postures shown in response to a fixed number of mounts (usually 10) x 100 (Beach, 1976).

Methods

I paired two couples, consisting of mature, sexually naive animals, in separate 15 gallon aquaria for 10 minutes a day over 10 consecutive days. Testing began between

0900 h-1000 h. Except during testing, the females were housed alone, and the males were housed together. Animals were placed in the aquarium on either side of a masonite divider for an acclimatization period of five minutes. At the end of this time, I raised the door in the middle of the divider to allow the animals access to one another. On days 3-10 of testing, the testing room was illuminated by only a 60 watt bulb to decrease the impact of the presence of the investigator. On days 7-10, in an attempt to make to testing situation more like the female's home cage, the female's bedding was put into the aquarium on the side in which she was acclimatized. Behaviors were recorded on a data sheet (Figure 13). Recorded behaviors were:

- female approach: female approached the male (subsequent behaviors imply male approach)
- male roll: male rolled onto his back underneath the female
- male sniff: male sniffed the ano-genital region of the female
- mount: male attempted to mount the female
- I: intromission
- E: ejaculation
- groom: both autogrooming and allogrooming were noted

These behaviors were recorded numerically in order of occurrence in 30 second time slots on the check sheet.

Behavioral Check Sheet: Paired Encounters

Date:

Time:

Observer:

Animal id: male: female:

Time	Female Approach	Male Roll	Male Sniff	Mount	I	E	Groom	Comments
:00								
:30								
1:00								
1:30								
2:00								
2:30								
3:00								
3:30								
4:00								
4:30								
5:00								
5:30								
6:00								
6:30								
7:00								
7:30								
8:00								
8:30								
9:00								
9:30								

Figure 13. Sample check sheet for scoring male-female interactions.

Female response was recorded following the numerical notation of each event using the following abbreviations:

- R: run away
- K: kick
- B: bite
- KT: kick threat, female raised leg but did not strike
- BT: bite threat, female bared teeth but did not strike
- P: posture, a specific response to male sniffing category in which the female raised her hindquarters

Results

Males showed initial interest by sniffing the females' ano-genital regions, but females usually responded by kicking the male or running away. Aggression in the form of biting was not seen. Interest shown by the male attenuated with time during the course of each trial. Mounting was never seen, and therefore female LQs could not be calculated. Lowering the lights decreased the animal's apparent awareness of the investigator. Adding the female's litter to the testing arena did not appear to affect the behavior of the female, however the male spent most of his time sifting through the litter, apparently foraging for oats.

Conclusions

Two possible interpretations of the results of this experiment are: 1) the females were not cycling, or 2) the animals were not exposed to each other at a time of day

conducive to mating. I did not examine the ovaries of these females, so I don't know whether they were cycling. If they were indeed cycling, it is possible that I did not see any mounting because I was not pairing the animals at the right time of day. In a subsequent experiment (described in Chapter IV), I examined the timing of copulation of eight mating couples, and found that copulations involving nulliparous females began from 0319 h-0600 h. In the present experiment, testing began after 0900 h, therefore the females may not have been physiologically ready to mate.

II.3.b Male Response To Female Urine

Female mammals advertise their readiness to mate via olfactory signals (Johnston, 1983), and the most likely source of such signals is urine, which contains the metabolic products of many hormones, as well as vaginal secretions. Hormone secretion from the gonads varies over the course of the reproductive cycle, and chemical contents of urine may vary with gonadal output (Johnston, 1983). If a male is receiving information about a female's reproductive state from her urine, his interest in the urine should fluctuate over the course of the female's estrous cycle.

Purpose

The objective of this study was to elucidate the estrous cycles of female *A. niloticus* by observing changes in male behavior in response to the daily presentation of female urine samples.

Methods

I collected daily samples of urine from eight mature female *A. niloticus*. Seven of these females were nulliparous (162-167 doa), and one had delivered one litter (174 doa).

Females were housed in metabolism cages, which had wire mesh bottoms that allowed all waste to fall through. The waste was collected in a test tube via a funnel that was suspended beneath the cage. Feces were kept separate from the urine sample by a plastic mesh screen placed in the bottom of the funnel, such that only liquid waste could pass through to the test tube. Females were fed powdered food from a cup that prevented the mixing of food with the urine sample. Once a week, the funnels and screens were washed and dried. At the termination of the experiment, the females were sacrificed, and their ovaries were removed and stored in 10% buffered formalin for future histological examination.

Urine samples were collected daily at 1700 h, and presented to eight males, along with a distilled water control, during a 20 min test every day for 21 d. Testing was done in a 15 gallon aquarium that was reduced to 1/3 floor space by a masonite divider, in order to retain the male in the area of the samples. I pipetted 200 µl of urine or water onto one inch squares of absorbent paper that were taped to the bottom of the test arena, about five inches apart. The placement of the samples was rotated, with water on the right on even numbered dates, and on the left on odd numbered dates. Testing was done in two runs of four males each, and all tests were videotaped for later analysis. There was no investigator in the testing room during testing. Videotapes were scored individually for each male, and the male's level of interest, as reflected in the amount of time the male spent sniffing and licking the urine sample, was recorded.

Results

The urine samples differed in quality between females. One female gave about 2 ml of light yellow colored urine every day, three females gave over 9 ml of light yellow colored urine, and three females gave 1-2 mls of urine which ranged in color from light brown to dark brown. The quality of urine did not appear to affect the male's level of interest.

Fluctuations of levels of interest were evident, but there did not appear to be any cyclic pattern (Figure 14). I thought that perhaps the females were not cycling, and upon histological examination of the ovaries, I found that four of them contained no corpora lutea, but that the other four (including the multiparous female) did display evidence of ovulation. Post-hoc analysis then revealed that the four males that had received urine from females who had ovulated showed greater fluctuations of interest than did the other males (Figure 14, A,C,G,H). Males exposed to urine from ovulating females showed peaks of interest with a range of 5-7 days between peaks.

Conclusions

These results were not significant, and although there is a trend that suggests that the cycles of the females involved in this study may have been somewhere between 5-7 days in length, there is no way to correlate the peaks in interest with an estrous cycle of with ovulation.

II.3.c Activity Rhythms

Changes in activity rhythms, such as an increase in activity, or phase advance of activity, have been associated with the estrous cycle in *Rattus norvegicus* (Wang, 1923),

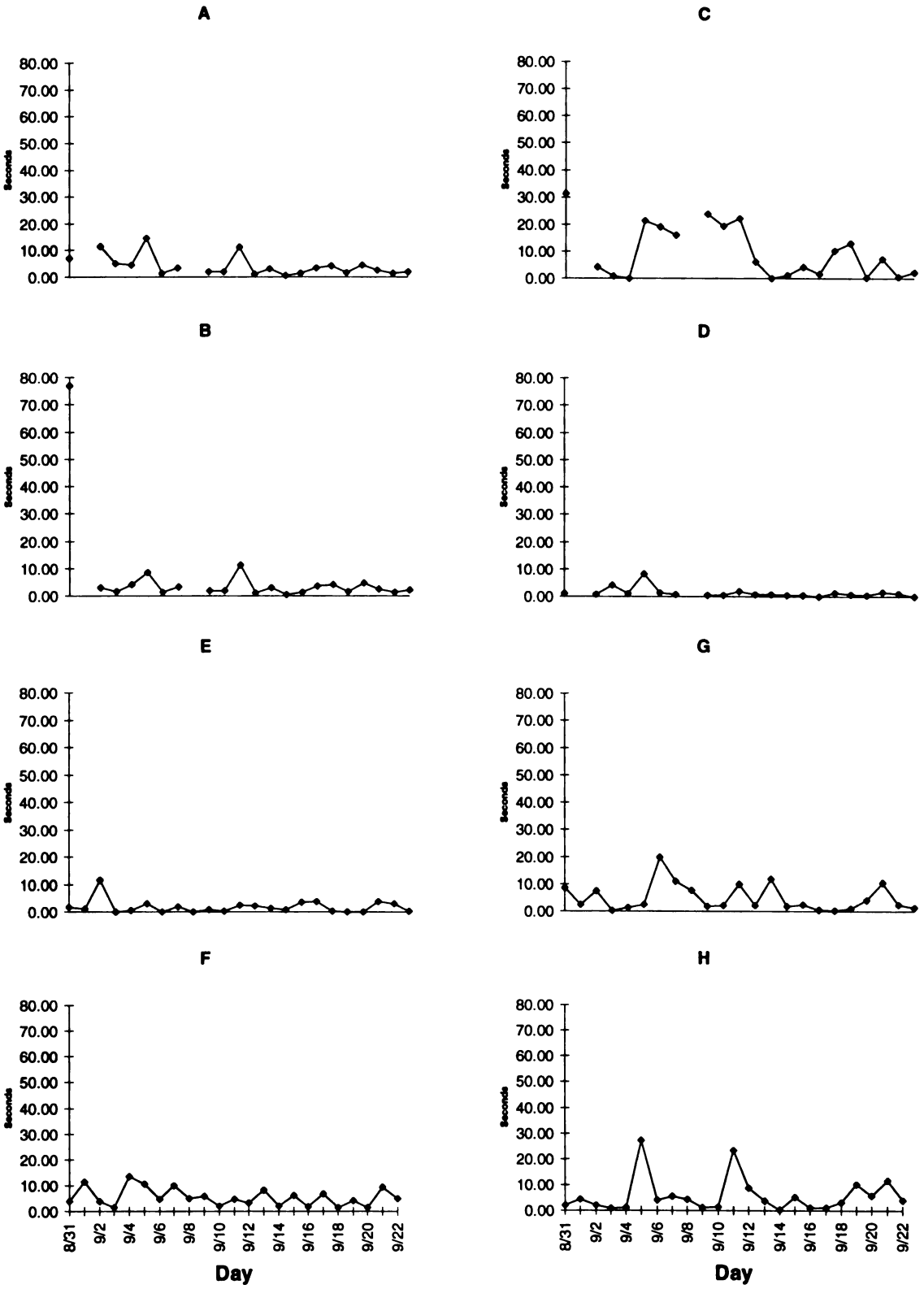


Figure 14. Male response to female urine sample represented by the number of seconds spent sniffing the sample.

Mesocricetus auratus (Zucker, 1980), and *Octodon degus* (Labyak and Lee, 1995). Activity peaks on the day of estrus in *R. norvegicus* (Wang, 1923). In *M. auratus*, activity is phase advanced, or begins earlier, by about 15 minutes on the day of estrus. *O. Degus* display both of the aforementioned patterns: on the day of estrus, activity onset is advanced two h, and shows an overall increase (Labyak and Lee, 1995).

Purpose

The purpose of this experiment was to elucidate the estrous cycle of *A. niloticus* by following the activity rhythms of several females over time.

Methods

Seven mature nulliparous female *A. niloticus* were implanted intraperitoneally with Mini-Mitter transmitters (Mini-Mitter, Inc., Sunriver, OR) to measure gross motor activity. Counts of activity were recorded as the transmitter moved over a wire grid in a receiver plate underneath the cage, and this information was relayed to a computer in an adjacent room. Data were collected in five minute intervals, or bins, using DataQuest III software (Mini-Mitter, Inc.).

Transmitter Implantation Surgery Protocol. Surgical instruments, autoclips, and cotton swabs were autoclaved before use. During the surgery, instruments were kept immersed in Nolvasan. Instruments were sterilized with 260°C dry heat between sequential surgeries with an Inotech dry sterilizer (model Steri 350). Animals were anesthetized with methoxyflurane (Metofane) in a glass chamber at the beginning of the procedure, and kept unconscious during the surgery using a nose cone filled with Metofane-soaked cotton. Surgeries were performed under a hood, and a heating pad was used to keep the

animal warm during the procedure. The abdominal area was shaved and wiped with Nolvasan, and an incision was made along the midline of the abdomen. Transmitters were kept immersed in Nolvasan for at least 1/2 h before surgery, and rinsed with sterile saline before insertion into the intraperitoneal cavity. Plain gut absorbable suture (5-0) with attached needle (FS-2) was used, and the muscle wall and the skin of the abdomen were sutured separately. Autoclips were used over the external sutures for reinforcement, and to prevent the animals from removing the sutures. The closed incision was covered with Nolvasan topical antibiotic cream (1% chlorhexidine acetate). The animal was placed in a normal tub cage with bedding and a 'house'. A heating pad was placed under half of the cage until the animal was completely recovered from the anesthetic. Following surgery, animals were treated with 1 cc of Lactated Ringer's solution and .01 cc buprenorphine hydrochloride (Buprenex) as an analgesic. For three days following surgery, animals received 12 mg of sulfamethoxazole and trimethoprim (SMZ-TMP), an oral antibiotic, in their drinking water. On the day of surgery, animals received softened rodent chow placed on the floor of the cage. For one week following surgery, rodent chow and oats were placed on the floor of the cage to prevent the animal from standing up to reach for food from the overhead bin, and putting strain on the incision. Autoclips were removed one week after surgery, and Nolvasan topical antibiotic cream was applied to the incision, which was checked for signs of infection. Incisions that were not completely healed, or that appeared off-color were flushed with Betadine surgical scrub (ammonium nonoxynol-4-sulfate), and Nolvasan topical antibiotic cream was applied.

Data from the same 31 day period were used for each of six animals. The daily records were scanned for evidence of activity onset. The mean daily level of activity

(measured as the number of grid wires crossed per time bin) was calculated for each individual, and these values were used to calculate overall mean values for the test period. Daily values were then plotted as standard deviations from the mean for each animal. The ovaries of the subject animals were prepared histologically as described above, and scanned for the presence of corpora lutea.

Results and Conclusions

There was no evidence of advancement of activity onset. There was no pattern of cyclic increases in activity, as illustrated by activity values plotted as standard deviations in Figure 15. Corpora lutea were present in the ovaries of females T1, T5, and T6, indicating that these animals had ovulated, but these ovulation events were not evident in the activity records. The results from this experiment showed that activity rhythms were not good indicators of the estrous cycle in female *A. niloticus*.

II.4 OVULATION

II.4.a Purpose

The results from the experiments described earlier in this chapter prompted me to look more closely at ovarian histology, in order to determine whether nulliparous females were indeed cycling. Therefore I conducted a study in which the objective was to determine whether the incidence of ovulation differs between nulliparous and multiparous female *A. niloticus*.

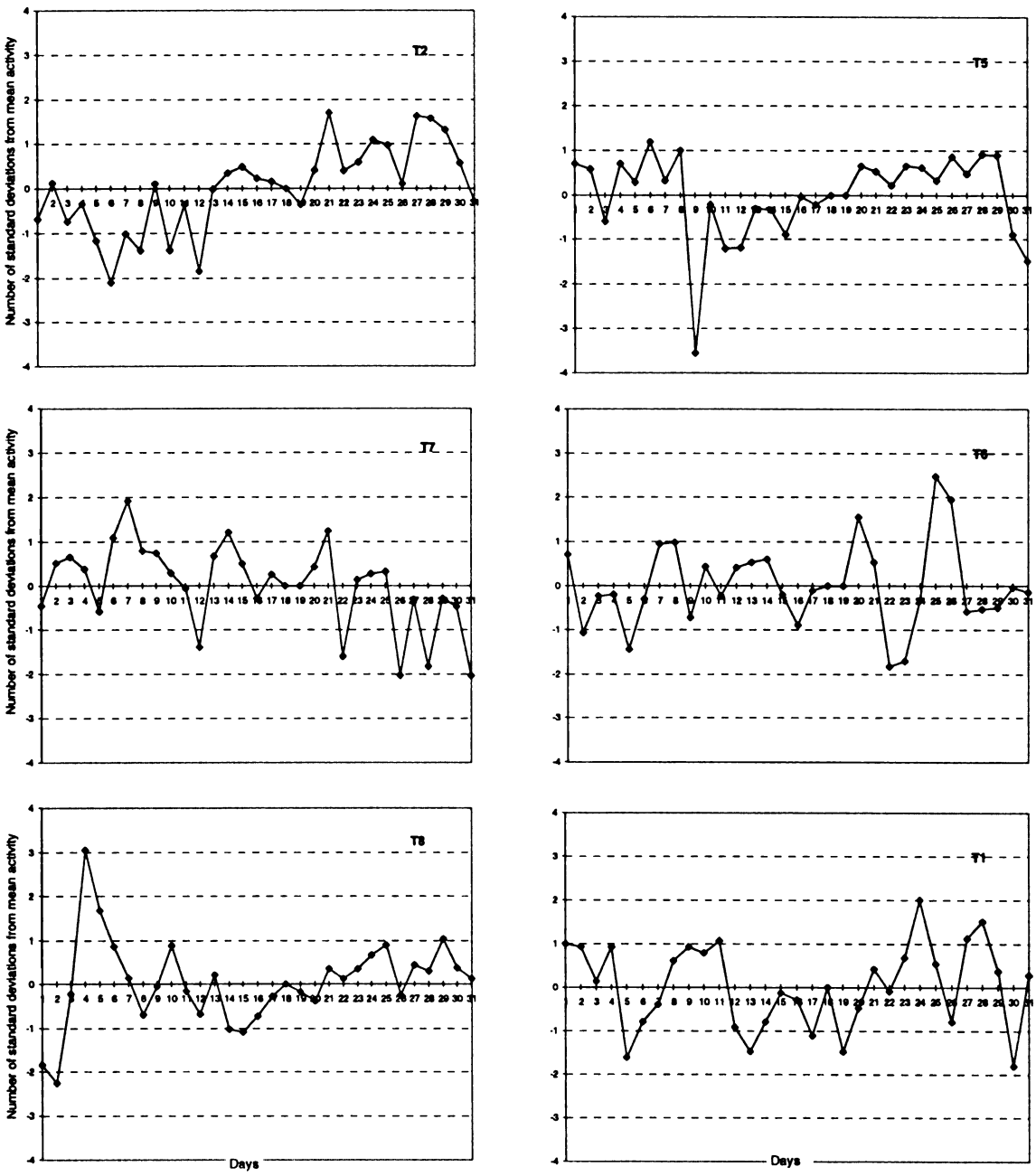


Figure 15. Number of standard deviations from mean activity measured over a period of 31 days.

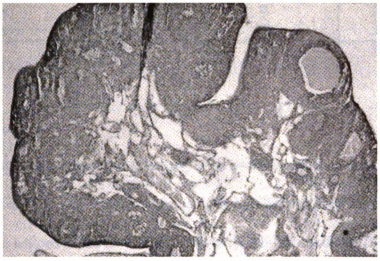
II.4.b Methods

I processed a total of 19 ovaries from multiparous females, and 59 ovaries from nulliparous females using the methods previously described. The multiparous females ranged in age from 155-580 d, and the nulliparous females ranged in age from 47-253 d. All but three of the multiparous females had been separated from their male partners for at least 50 days, and the three mating couples that remained together had stopped producing young. A chi-square test was performed to determine whether nulliparous females differed from multiparous females with regard to ovulation.

II.4.c Results

All of the ovaries from multiparous females contained corpora lutea, indicating that ovulation had occurred (Table 6, Figure 16B). Of the 59 nulliparous ovaries processed, only 10 exhibited evidence of ovulation, the remaining ovaries contained growing follicles, but no corpora lutea (Table 6, Figure 16A). Ovaries of multiparous females were significantly more likely to contain corpora lutea than were those of nulliparous females (X^2=42.442, df=1, $p<0.001$). Older (>100 doa) nulliparous females did not differ significantly from younger (<100 doa) females (two-tailed Fisher's exact test, p=0.476).

A



B

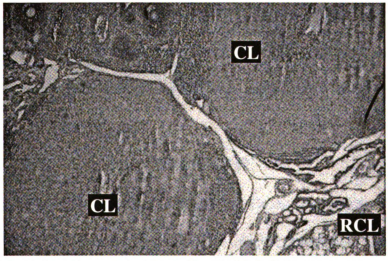


Figure 16. Nulliparous and multiparous ovaries of female *A. niloticus*. A nulliparous ovary (A), and a multiparous ovary (B), with 2 corpora lutea (CL) and one regressive corpus luteum (RCL). Note the size difference. These photos were shot at the same magnification (10X).

Table 6. Number of multiparous and nulliparous female *A. niloticus* exhibiting signs of ovulation.

	Corpora lutea present	Corpora lutea absent	n
Multiparous	19	0	19
Nulliparous	10	49	59
n	29	49	78

II.4.d Conclusions

The multiparous females in this study were spontaneous ovulators. Since they were not giving daily vaginal smears, there is no possibility that they were being induced to ovulate by the actions of the investigator. These results also indicate that most virgins that are isolated from males do not ovulate, as is also the case in the dwarf hamster, *Phodopus campbelli* (Erb *et al*, 1993). *P. campbelli* exhibits social control of puberty in that most females do not begin ovulatory cycling until they are exposed to a male. Female *A. niloticus* that did show evidence of ovulation in this experiment may have been induced to ovulate by olfactory cues emanating from males housed in the same room.

II.5 DISCUSSION

The estrous cycle of *A. niloticus* could not be elucidated by means of the vaginal cytological assay, behavioral assays, or monitoring of activity rhythms. Spontaneous ovulation occurred in all multiparous females that had been isolated from all conspecifics for 30 days. There is some indication that nulliparous females in this species may not begin ovulatory cycles when isolated from male conspecifics. This sort of social mediation of puberty is seen in the dwarf hamster (Erb *et al*, 1993). Delay of sexual

maturation in the female has also been seen in other rodents (reviewed by Levin and Johnston, 1986): *Mus musculus*, *Microtus californicus*, *M. ochragaster*, *Peromyscus maniculatus*, *Notomys alexis*, and *Meriones unguiculatus*. Levin and Johnston (1986) found a correlation between the lability of puberty onset and the degree of gregariousness of the species. Individuals of a gregarious species might be more likely to be influenced by the presence of conspecifics than would individuals of a species that live solitarily (Levin and Johnston, 1986).

Conaway (1971) questioned the importance of the non-pregnant estrous cycle in small rodents in natural populations. He referred to a cycle in which pregnancy does not occur "a pathological luxury which cannot be tolerated" (Conaway, 1971, p. 239). A non-pregnant estrous cycle is physiologically expensive, therefore it would be energetically efficient to delay cycling until such time as there is a high chance of becoming pregnant. While the results of the preceding experiments may be due to the stress of captivity, the hypothesis of delayed puberty merits further testing

CHAPTER III

THE MORPHOLOGY OF COPULATORY BEHAVIOR

III.1 INTRODUCTION

The pattern of behaviors that mammals display during copulation, or the morphology of copulation, varies widely among species, but is quite stereotyped among individuals within a species. Information on the morphology of copulation in a variety of species give us insight into the processes underlying the evolution of behavior and reproductive systems. However in this branch of the study of reproduction, as in the study of estrous cycles and ovulation, domesticated laboratory rodents are among the best-studied species; and domesticated animals are the least likely candidates to provide information on the relationship between behavior and ecological variables (Dewsbury,1972).

Dewsbury (1972) established criteria for the description of copulatory behavior in mammals, and outlined a process of study which involves three steps. The first step, classification, requires assigning a species to a category on the basis of the presence or absence of four behaviors: copulatory lock, intravaginal thrusting, multiple intromissions per ejaculation, and multiple ejaculations per copulatory series. A dichotomous tree of these four criteria results in 16 possible pattern types (Figure 17). The second step, elaboration, involves the quantitative description of the frequency and patterning of

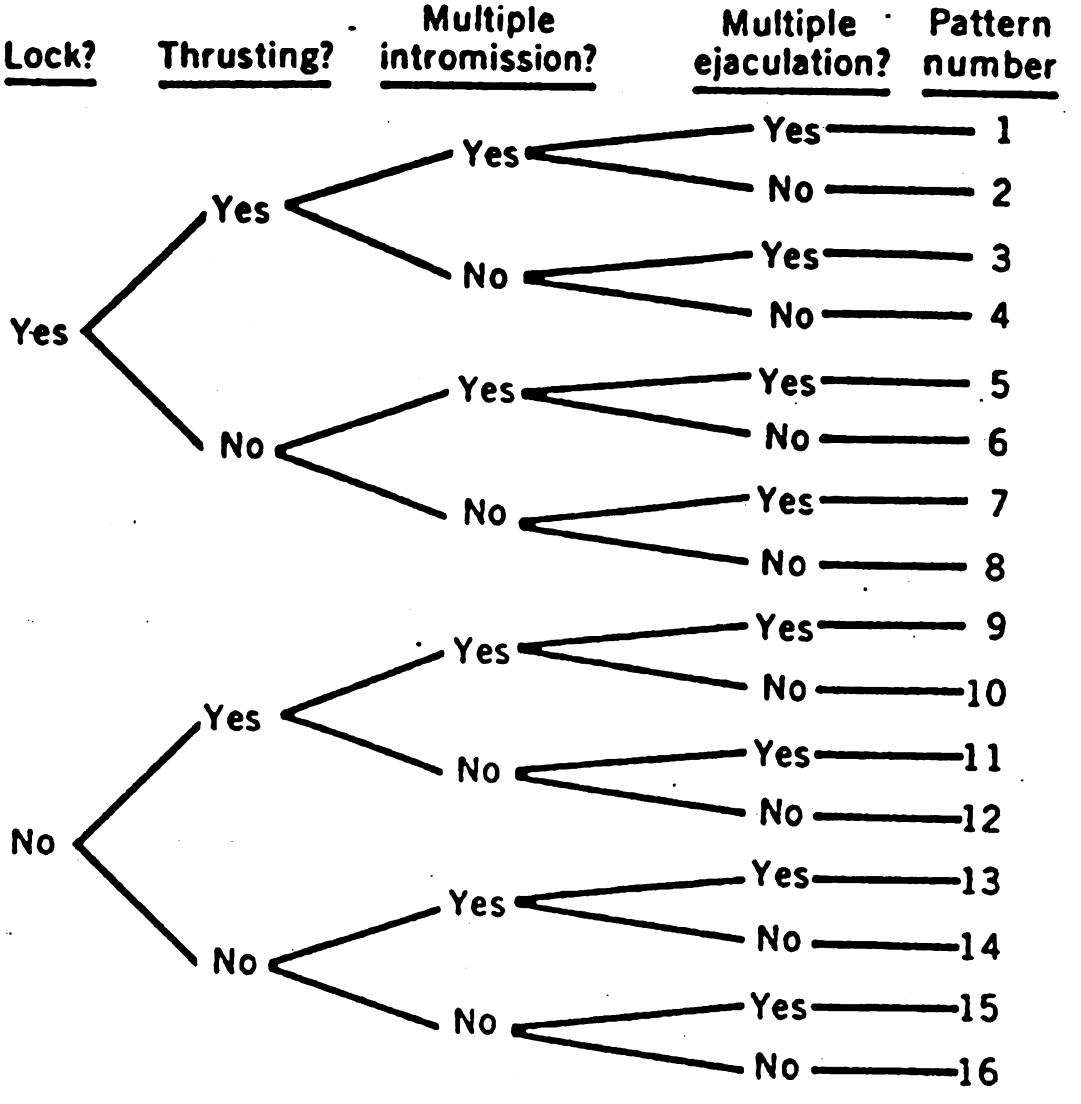


Figure 17. Patterns of mammalian copulation (from Dewsbury, 1972).

copulatory events, as well as the description of the conditions required for copulation, courtship behavior, and postures assumed during copulation. The third step involves experimental analyses of variables influencing copulatory behavior, such as the effects of genetics, experience, age and hormone levels. In this study of the morphology of copulation in *A. niloticus*, I will describe the classification of the species, and provide a quantitative description of the frequency and patterning of mounting and ejaculation.

III.2 METHODS

A total of fifteen mating couples of mature, sexually naive *A niloticus* were paired for use in this study, but only eight were actually used in data collection. Of the seven mating couples that were eliminated from the study, four never produced a litter, and three were excluded because of the death of one of the animals. Mating couples were housed in 15 gallon aquaria and filmed around the clock, using low-intensity red light for nighttime filming. In order to facilitate filming, animals were not given a house box, or refuge. Aquaria floors were covered with bedding, and food and water were available *ad libitum*. Filming was time-lapsed, recorded at 0.2 second intervals, such that 24 hours of film was recorded on two hours of tape. Four mating couples were filmed simultaneously, and the 15 mating couples were rotated through the video room over a 10 month period. Mating couples were removed from the video room at the cage change following the birth of their second litter. This filming schedule allowed the recording of the first (nulliparous) copulation, two births, and the post-partum copulations associated with these births.

When a litter was found, the videotape for the corresponding day was scanned, and the time of birth, as indicated by the appearance of the first pup, was recorded. Nulliparous copulations were found by subtracting 26 days (the longest estimation for gestation [Quilici *et al*, 1969]) from the birth date, and scanning videotapes in a reverse chronological order from that date. Post-partum copulations were found by scanning the videotape following the birth. In some cases, mating couples copulated a second time, as early as 15 d following post-partum copulations. These secondary copulations, in addition to the nulliparous and post-partum copulations, were scored using behavior check sheets (Figure 18), on which the frequency and patterning of copulatory events were recorded.

A total of 28 copulations were recorded: 8 nulliparous, 15 post-partum, and 5 secondary or multiparous. There was one case in which copulation did not occur post-partum, and one case in which a multiparous copulatory bout was not scored due to camera malfunction. I will describe only the nulliparous, multiparous and post-partum copulation bouts that resulted in fertile pregnancy, and the infertile post-partum copulatory events will be excluded.

The morphology of copulation in *A. niloticus* was described in accordance with Dewsbury's (1972) criteria. The number of mounts per ejaculation, and the duration of the postecjaculatory interval (PEI), defined as the interval between ejaculation and the resumption of copulation, were recorded for each ejaculation. This study also allowed me to establish the timing of several reproductive events. I calculated a definitive gestation for this species, and studied the timing of copulation and parturition (described in Chapter IV).

RAT SEX DATA SHEET

GRASS RAT COUPLE: DATE: TAPE STARTING TIME

_____ _____

MALE FEMALE OBSERVER

time	male chases female	female approaches male	male mounts female	groom (m/f)	male intromits	female lordoses	comments

Figure 18. Sample check sheet for scoring sexual behavior.

III.3 RESULTS

Copulation in this experiment consisted of the male chasing the female, who would lordose in response to a mount by the male. Copulation was easily recognized when scanning tapes due to its noticeable pattern, a circular chase interrupted by the short stops for mounting and ejaculation. The circular pattern of locomotion was probably due to the small size of the enclosures. There was no struggle or delay as males and females separated after intromission or ejaculation, so I concluded that *A. niloticus* do not display copulatory lock. From the time-lapse video, it did not appear that males were displaying intravaginal thrusting, and this was supported by direct observation of copulatory behavior in animals outside of this study. Male *A. niloticus* exhibited a series of shallow pelvic thrusts, a pattern which has been shown in the rat to be used to locate the opening of the vagina (Bermant, 1965). *A. niloticus* exhibited multiple intromissions per ejaculation, and multiple ejaculations per copulatory bout. This pattern of no lock, no thrust, multiple intromissions and multiple ejaculations can thus be assigned to pattern number 13 on Dewsbury's (1972) dichotomous tree (Figure 17).

It was impossible to determine whether an intromission was actually achieved during a mount, so only mounts and ejaculations were scored. In all but two cases, the number of mounts per ejaculation first decreased and then increased across a series of ejaculations within a copulatory bout(Figure 19). Length of the PEI increased with successive ejaculations within a copulatory bout (Figure 20).

Gestation ranged from 23.3-25.46 days (average 24.25 d). It appeared initially that some post-partum gestations were longer than nulliparous gestations, with a range of 30-52 d. However, by scanning the tapes 23-25 d before the date of the birth, I found

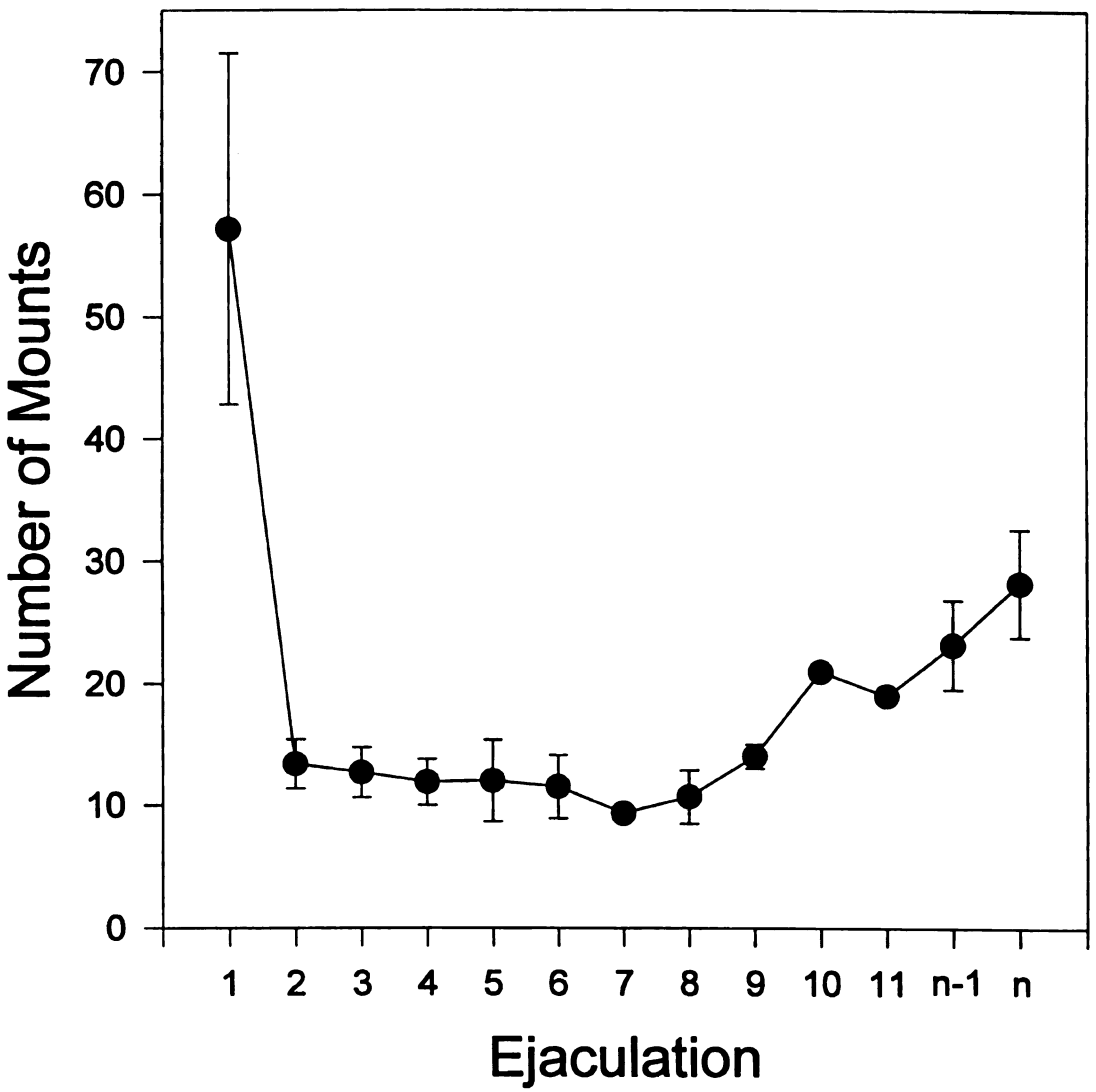


Figure 19. Number of mounts per ejaculation.

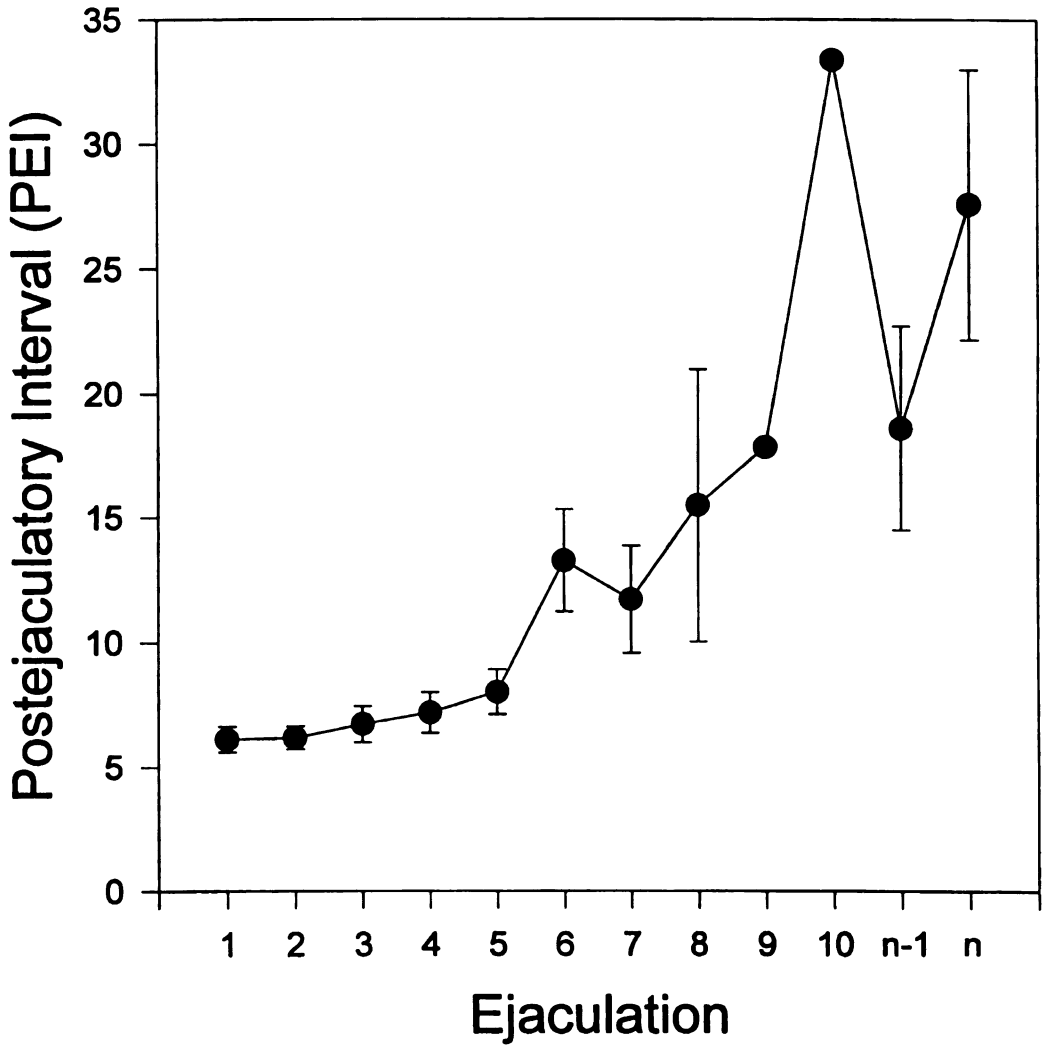


Figure 20. Postejaculatory interval per ejaculation.

that copulation was occurring a second time after post-partum copulation (secondary or multiparous copulations). Gestations resulting from these copulations were of durations comparable to the nulliparous gestations, and are included in the gestation range and average listed above.

III.4 DISCUSSION

The description of the copulatory morphology of *A. niloticus* on the basis of Dewsbury's four basic criteria allies this species with rodents in the genera *Rattus*, *Mesocricetus*, *Peromyscus* (the deer mouse), *Sigmodon* (the cotton rat), *Meriones* (jirds), and *Oryzomys* (the rice rat) (Dewsbury, 1975), which are murid and cricetid rodents. The decreasing, then increasing, pattern of mounting frequency over the course of a copulatory bout has been found in *Meriones unguiculatus*, *Oryzomys palustris*, and *Rattus norvegicus*. The pattern of increasing PEI length over the copulatory bout is seen in most rodents (Dewsbury, 1975).

This experiment resulted in the first accurate description of gestation for *A. niloticus*. The discovery of the secondary or multiparous copulations is significant. Ghobrail and Hodieb (1982) reported gestations of 35-51d for females suckling litters. The authors offered no hypothesis to explain the lengthened gestations (they reported gestations of 21-23 d for females not suckling litters). Without any further information, one might hypothesize that these lengthened gestations were the result of delayed implantation. However, in this experiment, I found that in apparent cases of prolonged gestation resulting from post-partum copulation, the animals actually copulated a second time. These secondary or multiparous copulations occurred from 15-28 d following the

post-partum copulations (in one instance in which the pups were eaten by the parents, copulation occurred five days after the post-partum copulation). If copulation and ovulation can occur 15 d after the birth of a litter of pups, lactation may be waning at this point. In *Rattus norvegicus*, females do not enter estrus until the young are weaned, at about 25-30 days (Nelson, 1995). Thus, perhaps weaning is taking place sooner in *A. niloticus* than the three week approximation reported by Delany and Monro from observations in the lab (1985a).

CHAPTER IV

TEMPORAL PATTERNS OF ACTIVITY, TEMPERATURE, AND REPRODUCTION

IV.1 INTRODUCTION

Biological rhythms synchronize the physiological, biochemical, and behavioral processes of an individual with environmental conditions that vary in a predictable rhythmic fashion. The physiological system that measures time and synchronizes these processes with daily events is known as the circadian timing system. The word circadian is used to describe the approximately 24-hour cycles that an organism endogenously generates (Moore-Ede *et al*, 1982)). Most research on the mechanisms controlling circadian rhythms in mammals has focused on a restricted number of animal models. Specifically, nocturnal rodents such as *Rattus norvegicus* and *Mesocricetus auratus* have been investigated extensively. Data on circadian rhythms and their neural substrates in diurnal mammals are relatively sparse, and we know nothing about how the substrates controlling circadian rhythms differ in nocturnal and diurnal species. One reason for this has been the lack of suitable diurnal rodent models with which to conduct experimental research.

As noted in Chapter I, reports from the literature regarding activity patterns in *A. niloticus* are conflicting, with the species described as diurnal (Delany and Kansiimeruhanga, 1970; Kingdon, 1974; Packer, 1983; Rabiu and Fisher, 1989), diurnal with crepuscular tendencies (Senzota, 1990), partially nocturnal (Ansell, 1960; Vesey-Fitzgerald, 1966; Harrison, 1972) and completely nocturnal (Schmutterer, 1969; Ghobrail and Hodeib, 1982). Data on the activity rhythms of *A. niloticus* in the field have been collected via direct observations (Harrison, 1972), trapping (Senzota, 1990; Rosevear, 1969) and shooting (Rosevear, 1969) records.

Evaluation of general activity rhythms of *A. niloticus* taken into captivity provides little clarification. Delany and Kansiimeruhanga (1970) reported the rhythm of only one animal. Duplantier and Granjon (1990) studied the rhythms of 11 individuals, but presented mean data for these animals, and did not describe rhythms from individuals. These two studies, the first of which describes only one subject, and the second of which describes the population rhythm, yield only an incomplete picture of activity in *A. niloticus*.

A. niloticus in our laboratory have displayed a range of diurnal and crepuscular wheel-running behavior (Katona and Smale, submitted); but the significance of wheel-running with respect to the behavior of animals in their natural habitat is unknown (Mather, 1981). Information about the general activity of a species aids in the study of its ecology. The adoption of a temporal niche of activity is as ecologically important to a species as its spatial niche (Moore-Ede *et al*, 1982), and this is particularly evident with respect to reproduction.

Reproductive events can be related to endogenous rhythms of activity. For example, receptivity in female *R. norvegicus* occurs predictably during the course of the 24 hour period (Keuhn and Beach, 1963; Hardy, 1972), and parturition in many mammalian species tends to occur at a specific time during the light-dark cycle (Jolly, 1973; Rowland et al, 1984). The timing of both receptivity and parturition are especially important in small-bodied, short-lived mammals. A female who is receptive during an active period is more likely to come across potential mates, and a female who gives birth during an inactive time is less likely to be disturbed by predators or conspecifics that may cannibalize her pups (Jolly, 1973).

The objective of this study was to determine whether the biological rhythms of wheel-running, gross motor activity, and core body temperature in *A. niloticus*, together with the timing of copulation and parturition, form a coordinated pattern, and if so, whether the pattern is nocturnal, diurnal, or crepuscular.

IV.2 ACTIVITY AND TEMPERATURE RHYTHMS

IV.2.a Purpose

The purpose of this experiment was to characterize the body temperature rhythm in this species, and to examine the relationship between rhythms in body temperature and rhythms in general activity and wheel running.

IV.2.b Methods

I used a combination of radio-telemetry transmitters and running-wheels to monitor activity and core body temperature. Seven mature virgin female *A. niloticus* were implanted intraperitoneally with Mini-Mitter telemetry transmitters to measure core body temperature and gross motor, or general, activity (for surgery protocol, see Chapter II). Three of the animals were placed in cages containing running wheels (26 cm diameter, 8 cm width), and four were left in standard cages without wheels. Data from the transmitters and the running wheels were recorded by a computer in an adjacent room using the DataQuest III data collection system (Mini-Mitter, Inc., Sunriver, OR) that stored information in five-minute bins. Each wheel revolution was recorded when a magnetic bar attached to the running-wheel strut closed a switch mounted on the wheel housing. A receiver plate was placed beneath each cage to relay the individual temperature and activity signals from each female's transmitter to the computer (Figure 21).

Here, I present data for each animal from a 10 day period beginning 3 weeks after surgery (with the exception of animal T3, for which we began data collection 9 weeks after surgery, due to technical difficulties). Histograms representing the average activity for each hour in a 24 h period bins were used to graphically analyze data for the description of daily rhythms in gross motor activity and core body temperature. Morning and evening peaks were evident in wheel-running, temperature, and gross motor activity patterns of each animal, and several features of these peaks were measured from 24 h histograms divided into 20-minute bins (see Methods: Activity and Temperature Rhythms). To evaluate the relationship between gross motor activity and core body

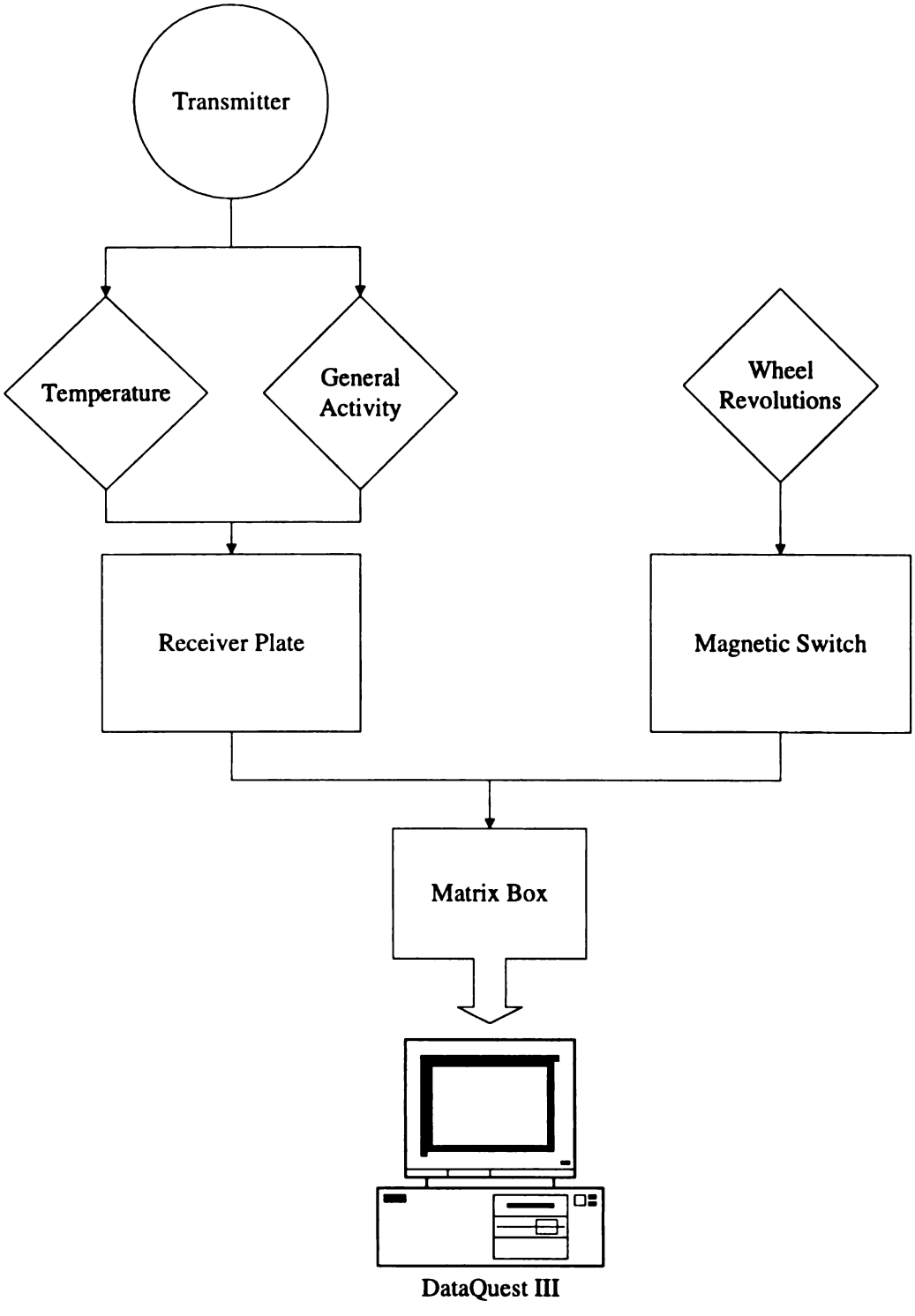


Figure 21. Data collection scheme for study of biological rhythms in *A. niloticus*.

temperature, the morning rises in activity and temperature were compared using histograms divided into 10-minute bins. In order to determine whether *A. niloticus* are diurnal with respect to general activity and body temperature, I compared the hourly values of these variables in the dark phase with the trough from the light phase, and I calculated the percent of one h bins which fell below the level of the light-phase trough.

IV.2.c Results

Daily patterns of wheel-running, gross motor activity, and body temperature of *A. niloticus* were extremely predictable and precise (Figure 22). The three animals with running-wheels were decidedly crepuscular in both general activity and wheel-running, with peaks immediately before the beginning, and after the end, of the light phase (Figures 22, 23). Individuals housed in cages without running wheels also displayed crepuscular peaks in general activity, but activity levels remained higher relative to the peaks during the light phase, than did activity in animals housed without running wheels.

Individuals housed in cages without wheels displayed absolute peaks in general activity from 0611 h-0729 h and at 1975 h, and individuals with running wheels displayed absolute general activity peaks from 0552 h-0650 h and 1926 h-1945 h. While there was variability among individuals with respect to level of activity, general activity peaked at higher levels in animals outfitted with running wheels than in animals housed in standard cages (Figure 23). Animals in standard cages displayed higher light phase trough levels than did animals with running-wheels, and dark phase trough levels were similar between the two groups (Figure 24).

Peaks in core body temperature were associated with peaks in general activity. As

Figure 22. Biological rhythms in female *A. niloticus*. Actograms of general activity, core body temperature and wheel-running activity of animals T1 and T6, divided into 10 minute bins, throughout ten 24 h periods.

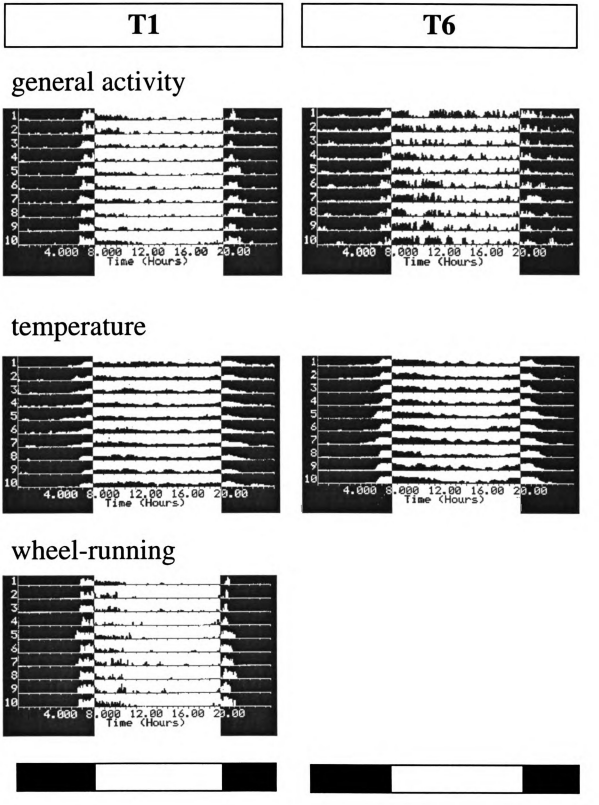

T1
T6
general activity
4.000 8.000 12.00 16.00 20.00
Time (Hours)
temperature
4.000 8.000 12.00 16.00 20.00
Time (Hours)
wheel-running
4.000 8.000 12.00 16.00 20.00
Time (Hours)

Figure 22.

Figure 23. A comparison of female *A. niloticus* with running-wheels (❍), vs. females without running-wheels (●) with respect to core body temperature and gross motor activity. Patterns of core body temperature (A) and gross motor activity (B), are divided into 1 h bins and averaged over 10 days.

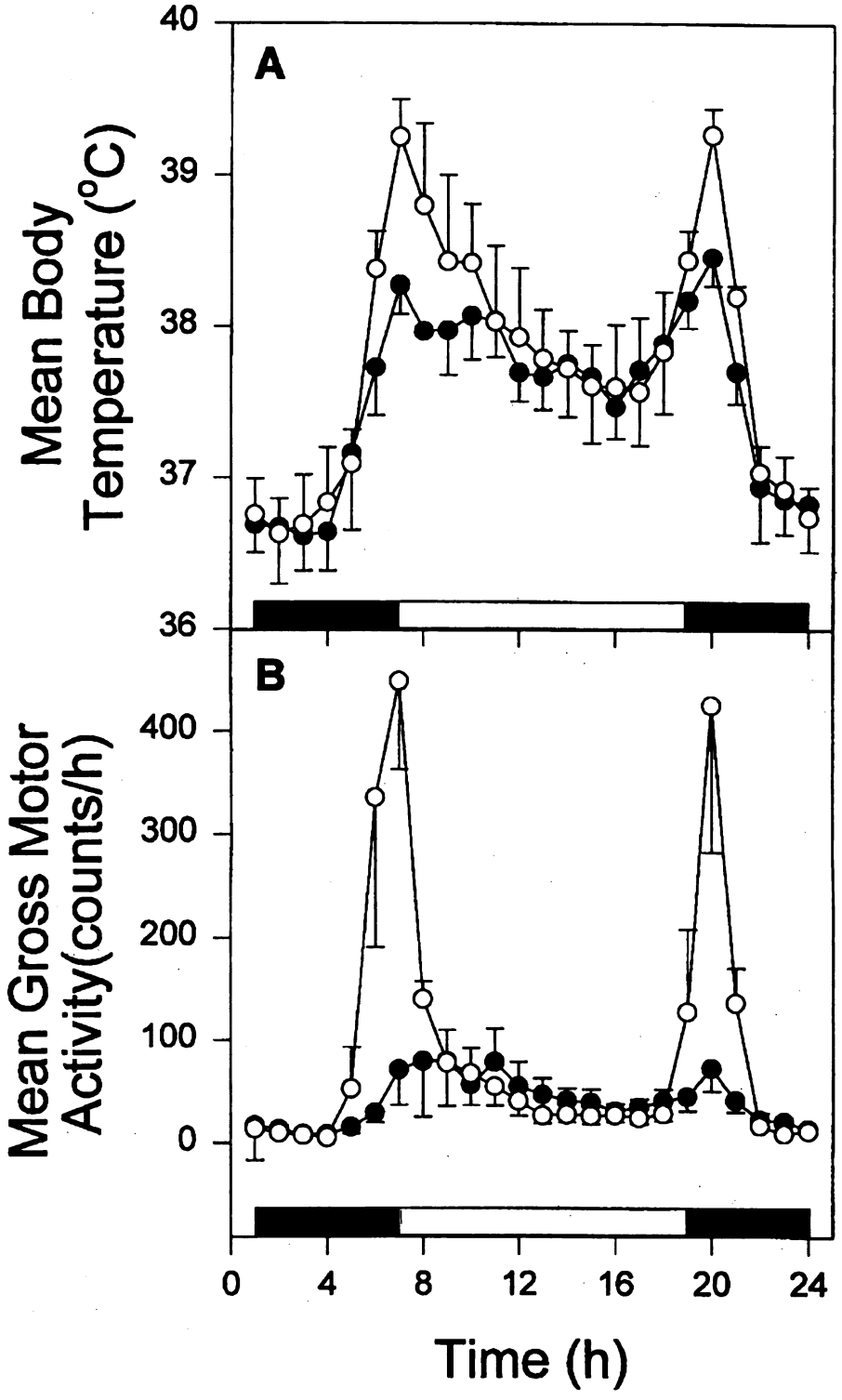

A
B
Mean Body Temperature (°C)
Mean Gross Motor Activity(counts/h)
Time (h)
40
39
38
37
36
400
300
200
100
0
0
4
8
12
16
20
24

Figure 23.

Figure 24. A comparison of patterns of gross motor activity and core body temperature in female *A. niloticus* with running wheels (B) vs. females without running wheels (A). Patterns of gross motor activity (○) and core body temperature (●), divided into 1 h bins and averaged over 10 days.

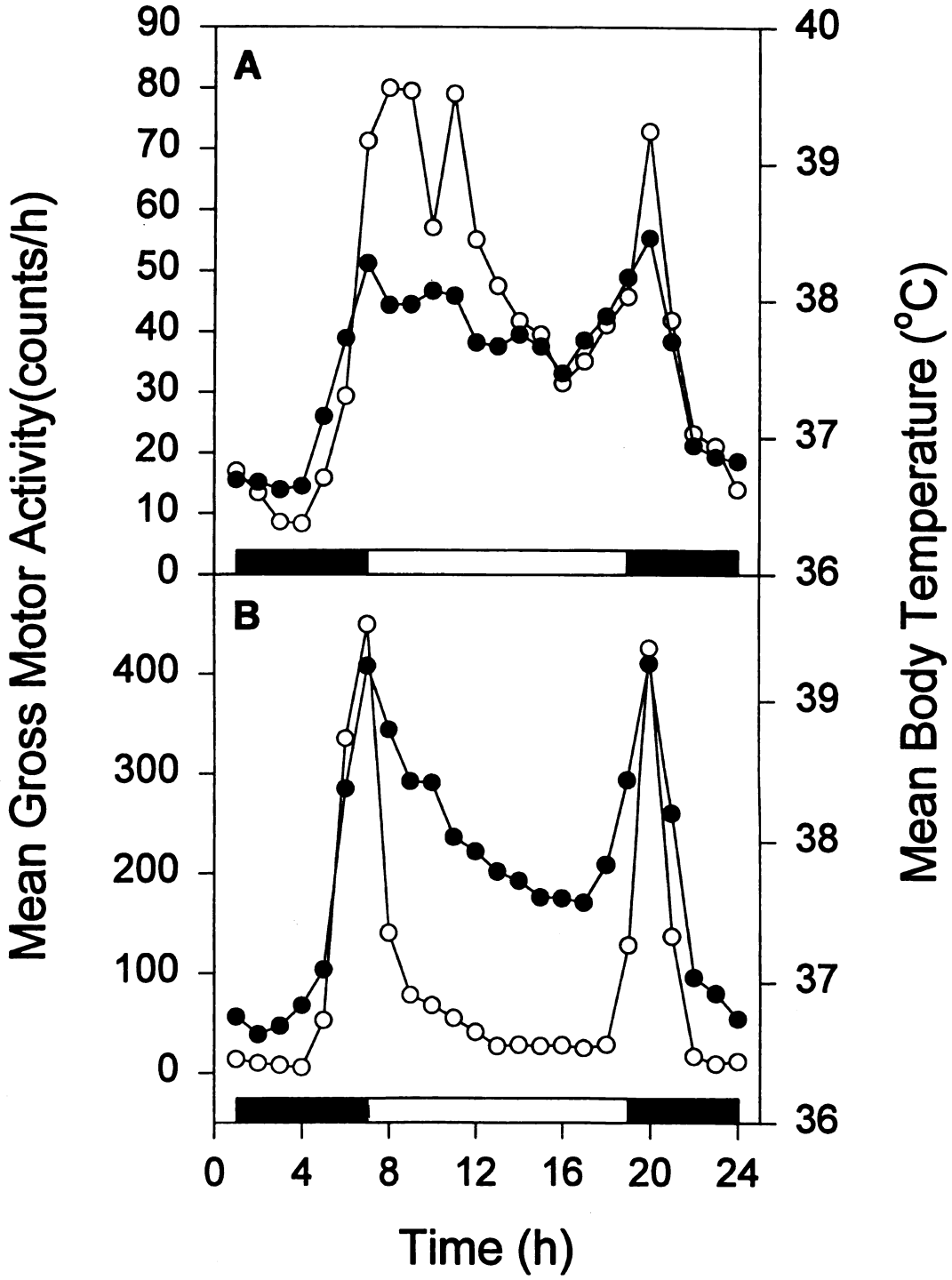

A
B
Mean Gross Motor Activity(counts/h)
Mean Body Temperature (°C)
Time (h)
0
10
20
30
40
50
60
70
80
90
100
200
300
400
36
37
38
39
40
0
4
8
12
16
20
24

Figure 24.

with general activity, peaks in temperature reached higher levels in individuals with running wheels (morning peak range: 38.45°C-39.69°C; evening peak range: 39.29°C-39.57°C) than in animals without running wheels (morning range: 38.09°C-38.9°C; evening range: 38.09°C-39.09°C) (Figure 23). Morning rises in activity occurred almost simultaneously with rises in core body temperature (Figure 25). This is especially clear in animals outfitted with running wheels (Figure 25: D, E, F).

In all seven animals, the majority of values for the 12 one-hour bins of activity in the dark phase were lower than the lowest value in the light phase (range: 58.33-75%) (Table 7). This trend was also observed for values of body temperature, although there was one female in which 50% of the values for the dark phase were below the light phase trough.

Table 7. Percentage of one-hour bins in the dark phase with mean values below those of the light phase trough.

Animal id	Activity	Temperature
T1	66.67%	66.67%
T2	58.33%	50.0%
T3	58.33%	66.67%
T5	66.67%	75.0%
T6	75.0%	75.0%
T7	75.0%	75.0%
T8	66.67%	66.67%

Figure 25. A comparison of the morning rise in gross motor activity and in temperature. The pattern of gross motor activity (❍) and core body temperature (●) from 0300h-0600h, are divided into 10 minute bins and averaged over 10 days, in females without running-wheels (A-D) and females with running wheels (E-G).

IV.3 TIMING OF COPULATION AND PARTURITION

IV.3.a Purpose

The purpose of this experiment was to characterize *A. niloticus* with respect to temporal organization of copulation and parturition.

IV.3.b Methods

Data from the study on the morphology of copulation (Chapter III) were used in this study. Mature, sexually naive males and females were paired in 15 gallon aquaria and filmed around the clock. Data were collected from 8 mating couples that produced litters during a ten month study period. Couples were filmed from the time of pairing through the birth of their second litter. Thus, initial sexual encounters, plus two births and two post-partum copulatory series were filmed for each couple. Pups were removed at 21 days. When a new litter was found, the entire videotape for that day was scanned, and the time of parturition (the sighting of the first pup) was noted. The time of initiation of copulation was recorded for three types of copulatory series: the first series that occurred for a given mating couple (nulliparous, n=8), post-partum copulation (n=15), and secondary copulation, which occurred if the post-partum copulation did not result in a successful pregnancy (multiparous, n=9).

Using data from the experiment on activity and temperature rhythms, peak periods of general activity were calculated for animals housed without wheels, and the timing of copulation and parturition of animals in this experiment were compared with these peaks, to determine whether these events were more likely to occur during times of activity or rest. The rise of each peak was defined as beginning at the lowest 20-minute bin before a

steady increase to the peak, and the end of the peak was defined as the lowest 20-minute bin following the peak. The starting and ending points of the morning and evening peaks of general activity were averaged to obtain times of peak activity. Using these periods of peak activity, the 24 hour day was split into active (total 5.09 h) and inactive (total 18.91 h) periods. The binomial test (Snedecor and Cochran, 1967) was used to test whether the distribution of timing reproductive events over these two periods was random.

IV.3.c Results

The eight copulations involving nulliparous females all began between 0319 h-0600 h (Figure 26). Six of the eight copulations were initiated during the morning period of peak activity. Postpartum copulations were initiated between 0511 h and 1937 h. A cluster of 12 copulations occurred between 0511 h and 0640 h (Figure 26). Of the remaining three instances of mating, one occurred at 0956 h, one at 1140 h, and the last at 1937 h. There was only one instance in which a mating couple did not copulate post-partum. The parturition to post-partum copulation interval ranged from 0216 h-2024 h (n=15). Interestingly, the majority of post-partum copulation (n=9) events were not fertile. Fertile post-partum events (n=6) occurred with a parturition to post-partum copulation interval range of 0216 h-1124 h.

When the first post-partum mating event was infertile, second bout of mating occurred between 15 to 28 days after the first post-partum copulation. In one case, there was no nighttime video due to camera malfunction, so information on initiation time was not available for that bout of copulation. However these animals were found mating when room lights came on. In the remaining five cases, copulation occurred between

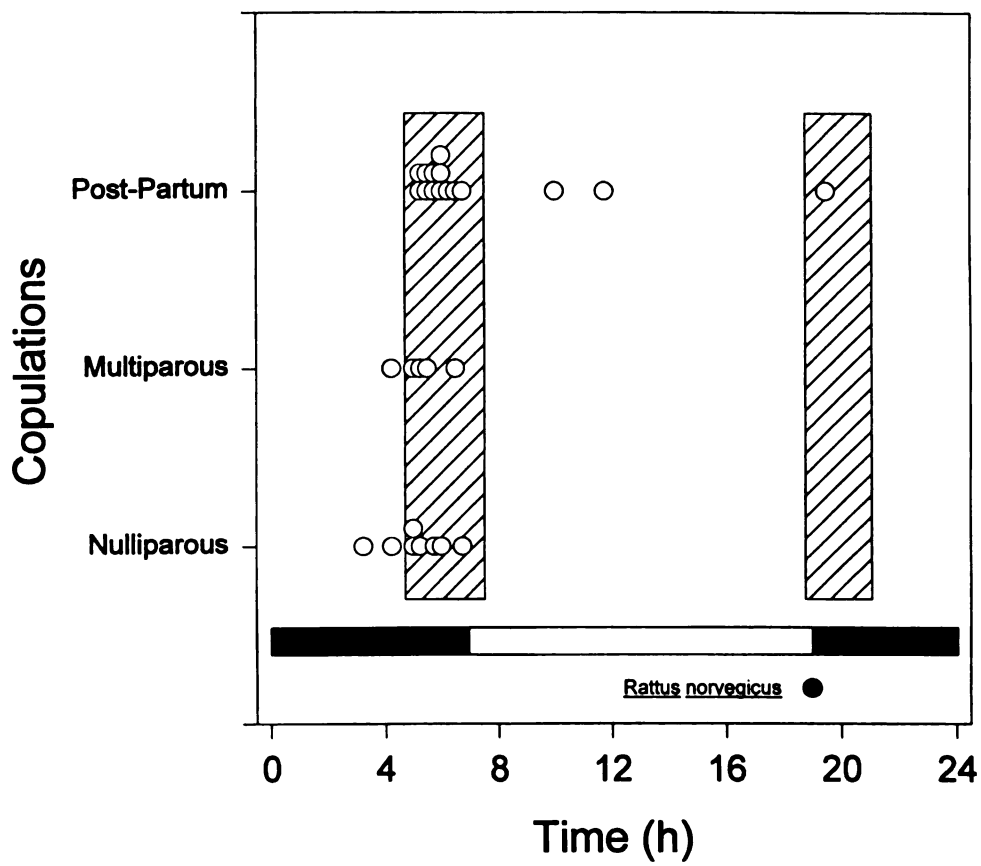


Figure 26. Timing of copulation in captive *A. niloticus*. The onset of 28 copulations in *A. niloticus* (○), and the beginning of heat in *R. norvegicus* (●), after Hardy, (1972) plotted over the peak periods of general activity (indicated by the hatched bars).

0411 h and 0633 h (Figure 26).

Of the total of 28 copulations observed, 23 began during times of peak activity as defined with the general activity data. The probability of this distribution occurring by chance alone (P=0.50) is p=.0024.

The 16 recorded parturitions occurred in two clusters, one during the light phase from 1025 h-1516 h (five cases), and the other during the dark phase from 1945 h-0410 h (11 cases) (Figure 27). Sixty-nine percent of the births occurred during the dark period, and 87.5% occurred outside the periods of peak activity. The probability of this distribution occurring by chance alone (P=0.50) is p=0.016.

IV.4 DISCUSSION

A. niloticus has previously been shown to be diurnal and crepuscular with respect to wheel-running activity (Katona and Smale, in press). Further exploration of biological rhythms in this species has revealed that *A. niloticus* is a diurnal mammal, and displays a suite of adaptations to this general pattern, involving general activity, sexual activity, and temperature. In this experiment, wheel-running was crepuscular. Previous wheel running experiments with this species showed some individuals to be diurnal, and others crepuscular (Katona and Smale, in press). Patterns of general activity and temperature were both diurnal, although peaks occurred just before the start of the light phase, and just after the beginning of the dark phase. Levels of general activity and temperature remained higher during the light phase than in the dark phase, giving both patterns a strong diurnal component. The general pattern of activity seen in *A. niloticus* of two peaks separated by a trough is the most common among wild animals, and is different

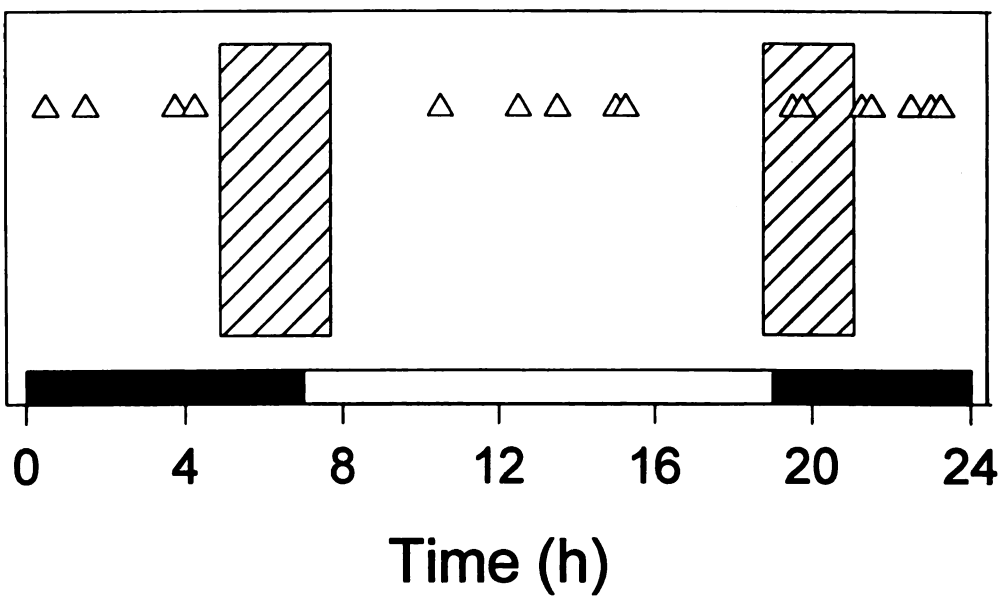


Figure 27. Timing of parturition in captive *A. niloticus*. Time of beginning of parturition (Δ) plotted over the peak activity periods represented by the hatched bars.

from the one-peak pattern seen in most laboratory animals (Aschoff, 1966). Thus the peaks seen in *A. niloticus* do not warrant the species' classification as crepuscular, but rather as a diurnal animal with crepuscular tendencies.

It is widely believed that, although high levels of activity can cause slight elevations in body temperature, the circadian rhythm of body temperature is not simply a secondary effect of the circadian rhythm of activity. One reason that investigators have come to this conclusion is that in humans and many animals, core body temperature starts rising before the individual awakens and becomes active (Refinetti and Menaker, 1992). One of the most interesting findings of this study is that in *A. niloticus*, temperature rises in concert with gross motor activity in the morning.

In *R. norvegicus*, heat, signified by the display of lordosis and low incidence of rejection of the male, begins around dusk, and females are maximally receptive for six to nine hours (Keuhn and Beach, 1963; Hardy, 1972) thus female *R. norvegicus* are receptive during their periods of peak activity. Similarly, of the 28 instances of mating recorded in *A. niloticus*, all but six (78.6%) began during the narrow window of the morning peak of activity, 0440h-0729h . Of the six mating bouts that did not occur during this time, four were closely associated with one of the two daily peaks of activity. Three of these copulation bouts began within 1.5 h before the morning rise in activity at 0440h, and one began during the evening activity peak. Thus, in both *R. norvegicus* and *A. niloticus*, mating occurs at the beginning of the active phase, but these species are opposite one another in phase. In *R. norvegicus*, copulation occurs at dusk, whereas it occurs just before dawn in *A. niloticus.*

Diurnal New and Old World monkeys give birth at night, while nocturnal prosimians deliver during the day (Jolly, 1973). The nocturnal species *R. norvegicus* and *M. auratus* tend to deliver their young during the light period (Mitchell and Yochim, 1970; Lincoln and Porter, 1976; Connor and Davis, 1980; Viswanathan and Davis, 1992), which represents their inactive phase. In *R. norvegicus*, it is the biological rhythm of the mother that determines the time of birth (Lincoln and Porter, 1976). Female *A. niloticus* delivered 68.75% of their litters during the dark phase, and 87.5% of all litters were delivered outside of periods of peak activity. Thus, the timing of parturition in *A. niloticus* appears to exhibit a pattern that might best be described as anti-crepuscular.

In *R. norvegicus,* timing of post-partum copulation depends upon the timing of parturition, and is indirectly dependent on the time of day in that parturition usually occurs during the light phase (Connor and Davis, 1980). In *R. norvegicus*, females display lordosis in response to manual stimulation 4-36 h after parturition (Blandau and Soderwall, 1941), and display peak receptivity to an active male an average of 12 h after parturition (Connor and Davis, 1980). *A. niloticus* are different from *R. norvegicus* with respect to the timing of post-partum copulation in two ways. First, *A. niloticus* are capable of copulating much sooner after parturition than are *R. norvegicus*. The shortest parturition to copulation interval that resulted in pregnancy recorded here for *A. niloticus* was 0216 h, and all fertile post-partum copulations began earlier than the average peak receptivity seen in *R. norvegicus* at 12 h post-partum. *A. niloticus* may also differ from *R. norvegicus* with respect to the timing mechanism controlling post-partum copulation. Twelve of the 15 instances of post-partum copulation in *A. niloticus* began during the morning peak. However, in contrast to the findings of Connor and Davis (1980), this

clumping of post-partum copulation times in *A. niloticus* was not a simply the result of clumping of parturition times. Female *A. niloticus* appeared to be 'waiting' until their period of peak activity in order to copulate. Recall, however, that most of these *A. niloticus* copulation events (9/15) did not result in fertile pregnancy. Although we initially ascribed this low post-partum success rate to a problem with pacing (the female had no place to take refuge from the male), it may be that the experimental conditions caused some anomaly in timing. Perhaps the reason that the copulations did not result in pregnancy was that the females waited until the next morning to copulate.

In conclusion, the rhythms of *Arvicanthis niloticus* are predictable, clear, and most certainly diurnal. This species is an excellent candidate for a diurnal model with which to study circadian rhythms. The bimodal activity pattern displayed by *A. niloticus* are more typical of wild animals than of other lab rodents.

CONCLUSIONS AND RECOMMENDATIONS

Conventional means for elucidating the estrous cycle in rodents have failed with *Arvicanthis niloticus.* Although two lines of evidence suggested the existence of a 5-7 day cycle length, the search for an estrous cycle in this species revealed no conclusive evidence. It is possible that the non-pregnant estrous cycle may be unimportant to female *A. niloticus* in the wild. In a rodent that exhibits a post-partum estrus, the non-pregnant estrous cycle may be obsolete, with conception occurring at each post-partum estrus during the breeding season. Captive *A. niloticus* females are easily brought into behavioral estrus with the priming protocol of estradiol and progesterone injections (Carmen Salsbury, personal communication) used for *R. norvegicus*. Therefore, future studies that require an estrous female could use primed subjects, and the need for predicting the time of natural estrus is reduced.

Regardless of whether the non-pregnant estrous cycle occurs in the wild, the question of ovulation in captive *A. niloticus* requires further study. One area of research that deserves deeper investigation is that of the nature of the corpus luteum. Knowing the lifespan of the corpus luteum in *A. niloticus,* and its cycle of degeneration, would allow us to determine when a female ovulated, and to speculate how the female ovulated: spontaneously, or in response to some form of stimulation. Knowing more about the nature of the corpus luteum would also be helpful in other studies regarding the

reproductive biology of female *A. niloticus*. I suggested in Chapter II that puberty might be delayed in females that are not exposed to males. A simple experiment would determine whether this is true. Comparison of the ovaries of females housed alone at weaning with those of females housed with male conspecifics would address this question.

A. niloticus display a diurnal activity pattern which is quite different from the nocturnal pattern of their well-studied cousins in the genus *Rattus*. The other six murid rodents within Kingdon's (1974) Arvicanthis division (see Chapter I), *Dasymys*, *Mylomys*, *Hybomys*, *Rhabdomys*, *Lemniscomys*, and *Pelomys*, all show at least some component of diurnality in their activity rhythms (Kingdon, 1974). Perhaps diurnality evolved within this group as it developed separately from that of other murid taxa. Comparative study using *A. niloticus* and *R. norvegicus* as diurnal and nocturnal models would greatly benefit the study of circadian rhythms, and possibly the study of the evolution of rhythms.

The utilization of *Arvicanthis niloticus* as a laboratory model opens new doors in the study of behavior. *A. niloticus* offer three unique characteristics to laboratory study. The first is that they have been recently brought into captivity. When studying domesticated animals like *Rattus norvegicus* and *Mesocricetus auratus*, one has to wonder whether the results are applicable to natural rodent populations. The second exceptional quality of *A. niloticus* is that they are diurnal, and a reliable diurnal rodent model is lacking. The third attribute that makes *A. niloticus* stand out is the gregariousness of the species, providing opportunity for the study of social behavior in the lab.

APPENDICES

APPENDIX A

Embedding Protocol for Paraffin Sections

50% EtOH	30 min
70% EtOH	30 min
95% EtOH	30 min
95% EtOH	30 min
95% EtOH	30 min
100% EtOH	30 min
100% EtOH	30 min
100% EtOH	30 min
Xylene	30 min
Xylene	30 min
Paraffin	30 min
Paraffin	30 min

APPENDIX B

Staining Protocol for Paraffin Sections

Hemo De	15 min
Hemo De	3 min
100% EtOH	2 min
100% EtOH	2 min
95% EtOH	2 min
70% EtOH	2 min
dH_2O	2 min
hematoxylin	5 min
dH_2O	dip
eosin	1 min
70% EtOH	dip
95% EtOH	dip
100% EtOH	dip
100% EtOH	dip
Hemo De	5 min
Hemo De	5 min

All alcohol was diluted with double distilled water.

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